Supplemental materials for the article:

# 4MRNA: a new approach for nucleic acid molecular replacement using models with diverse parameter patterns

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## Contents

## Supplemental methods



## Supplemental references

**Script S1:** The R script to make a rejection table for the Smirnov-Grubbs test. This is the code for a significance level of 0.05, with *n* ranging from 3 to 1000. By appropriately adjusting the value of *n*, it can handle data with a larger sample size.

```
Grubbs <- function(n, alpha) {
         t <- qt(1-alpha/n, n-2)^2
         return((n-1)*sqrt(t/(n-2+t))/sqrt(n))
}
n <- c(3:1000)
alpha <- c(0.05)
tbl <- outer(n, alpha, Grubbs)
rownames(tbl) <- n
colnames(tbl) <- alpha
tbl
```

**Script S2:** The Python code for model building used to investigate the effects of six helical parameters on molecular replacement with PDB ID 1IH1.

```
import os
import time
import shutil
import itertools
from selenium import webdriver
from selenium.webdriver.common.by import By
from selenium.webdriver.support.ui import WebDriverWait
from selenium.webdriver.support import expected_conditions as EC

download = ''              # Enter the default download directory for Microsoft Edge.
directory = ''             # Enter the working directory.
sequence = 'GGCGCC'        # This is the sequence of 1IH1.

def create_dna_model(text, download, directory):
    driver = webdriver.Edge()
    try:
        driver.get('http://web.x3dna.org/index.php/rebuild')

        WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="headnav5"]'))).click()

        WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="main"]/div[2]/table[1]/tbody/tr[4]/td[4]/input'))).click()

        pdb_id = text.replace("U", "T")

        pdbid_element = WebDriverWait(driver, 10).until(EC.presence_of_element_located((By.XPATH,
'//*[@id="pdbid"]')))

        pdbid_element.clear()
        pdbid_element.send_keys(pdb_id)

        WebDriverWait(driver, 180).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="formdiv"]/form/input[5]'))).click()
```

```
        time.sleep(10)

        WebDriverWait(driver, 60).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="content"]/div[1]/div[2]/a[2]'))).click()

        WebDriverWait(driver, 30).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="headnav1"]'))).click()

        time.sleep(5)

        file_path = f'{download}/fiber_model.pdb'

        file_input = WebDriverWait(driver, 10).until(EC.presence_of_element_located((By.XPATH,
'//*[@id="file"]')))

        file_input.send_keys(file_path)

        WebDriverWait(driver, 180).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="main"]/div[2]/table/tbody/tr/td[3]/form/input[2]'))).click()

        time.sleep(10)

        WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="content"]/div[3]/table[2]/tbody/tr/td/p[1]/a[2]'))).click()

        time.sleep(5)

    except Exception as e:
        print(f"Error: {e}")
    finally:
        driver.quit()
    time.sleep(5)
    source = f'{download}/bp_step.txt'
    shutil.move(source, directory)
    time.sleep(5)

def generate_all_combinations(adjustments, length):
    return list(itertools.product(adjustments, repeat=length))
```

```
def process_file(input_file, output_dir, adjustment_index, adjustments, label, offset=0):
    with open(input_file, 'r') as f:
        lines = f.readlines()

    header = lines[:4]
    data_lines = lines[4 - offset:]
    all_combinations = generate_all_combinations(adjustments, len(data_lines))
    for i, combo in enumerate(all_combinations, 1):
        output_lines = header.copy()
        for line, adjustment in zip(data_lines, combo):
            parts = line.split()
            if len(parts) > adjustment_index:
                try:
                    value = float(parts[adjustment_index])
                    new_value = value + adjustment
                    parts[adjustment_index] = f"{new_value:.2f}"
                    output_lines.append(' '.join(parts) + '¥n')
                except ValueError:
                    output_lines.append(line)
            else:
                output_lines.append(line)
        output_file = os.path.join(output_dir, f"bp_step_{label}{i}.txt")
        with open(output_file, 'w') as f:
            f.writelines(output_lines)
    return len(all_combinations)
create_dna_model(text, download, directory)
input_file = os.path.join(directory, 'bp_step.txt')

adjustments_dict = {
    'Buckle': [-14.84, 0, 14.84],
    'PropTw': [-15.42, 0, 15.42],
    'Opening': [-8.88, 0, 8.88],
    'Tilt': [-6.66, 0, 6.66],
    'Roll': [-10.82, 0, 10.82],
    'Twist': [-11.44, 0, 11.44]
}

tilt_files = process_file(input_file, directory, adjustment_index=10,
adjustments=adjustments_dict['Tilt'], label='Tilt')
```

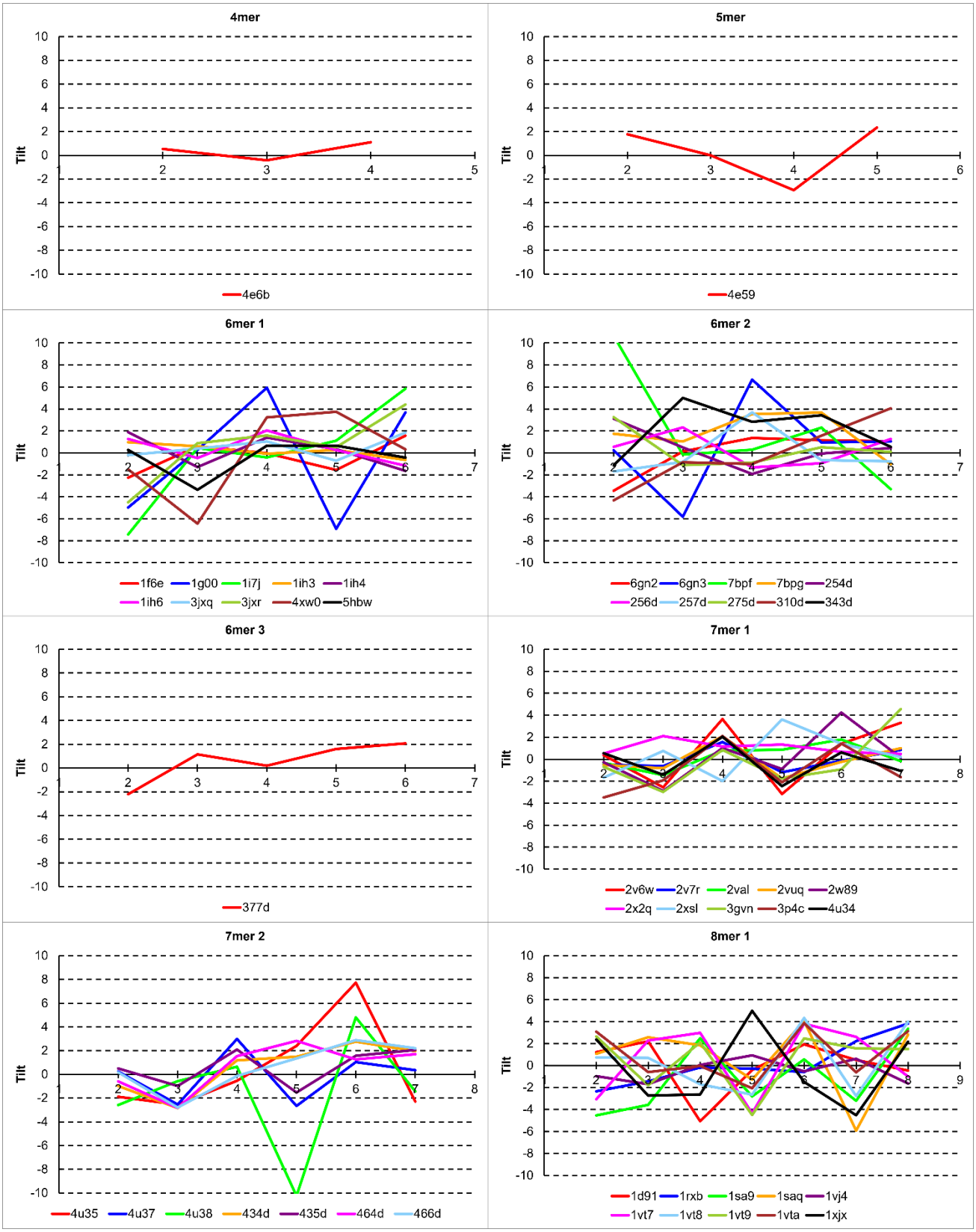
```
roll_files = process_file(input_file, directory, adjustment_index=11,
adjustments=adjustments_dict['Roll'], label='Roll')

twist_files = process_file(input_file, directory, adjustment_index=12,
adjustments=adjustments_dict['Twist'], label='Twist')

buckle_files = process_file(input_file, directory, adjustment_index=4,
adjustments=adjustments_dict['Buckle'], label='Buckle', offset=1)

prop_tw_files = process_file(input_file, directory, adjustment_index=5,
adjustments=adjustments_dict['PropTw'], label='PropTw', offset=1)

opening_files = process_file(input_file, directory, adjustment_index=6,
adjustments=adjustments_dict['Opening'], label='Opening', offset=1)

for filename in os.listdir(directory):
    if filename.endswith(".txt"):
        file_path = os.path.join(directory, filename)
        driver = webdriver.Edge()
        try:
            driver.get('http://web.x3dna.org/index.php/rebuild')

            WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="custom1"]'))).click()

            WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="r1"]'))).click()

            file_input = WebDriverWait(driver, 10).until(EC.presence_of_element_located((By.XPATH,
'//*[@id="formdivre"]/p[6]/input')))

            file_input.send_keys(file_path)

            WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="buildmodel"]'))).click()

            download_link = WebDriverWait(driver, 10).until(EC.element_to_be_clickable((By.XPATH,
'//*[@id="content"]/div[1]/div[2]/a[2]')))
```

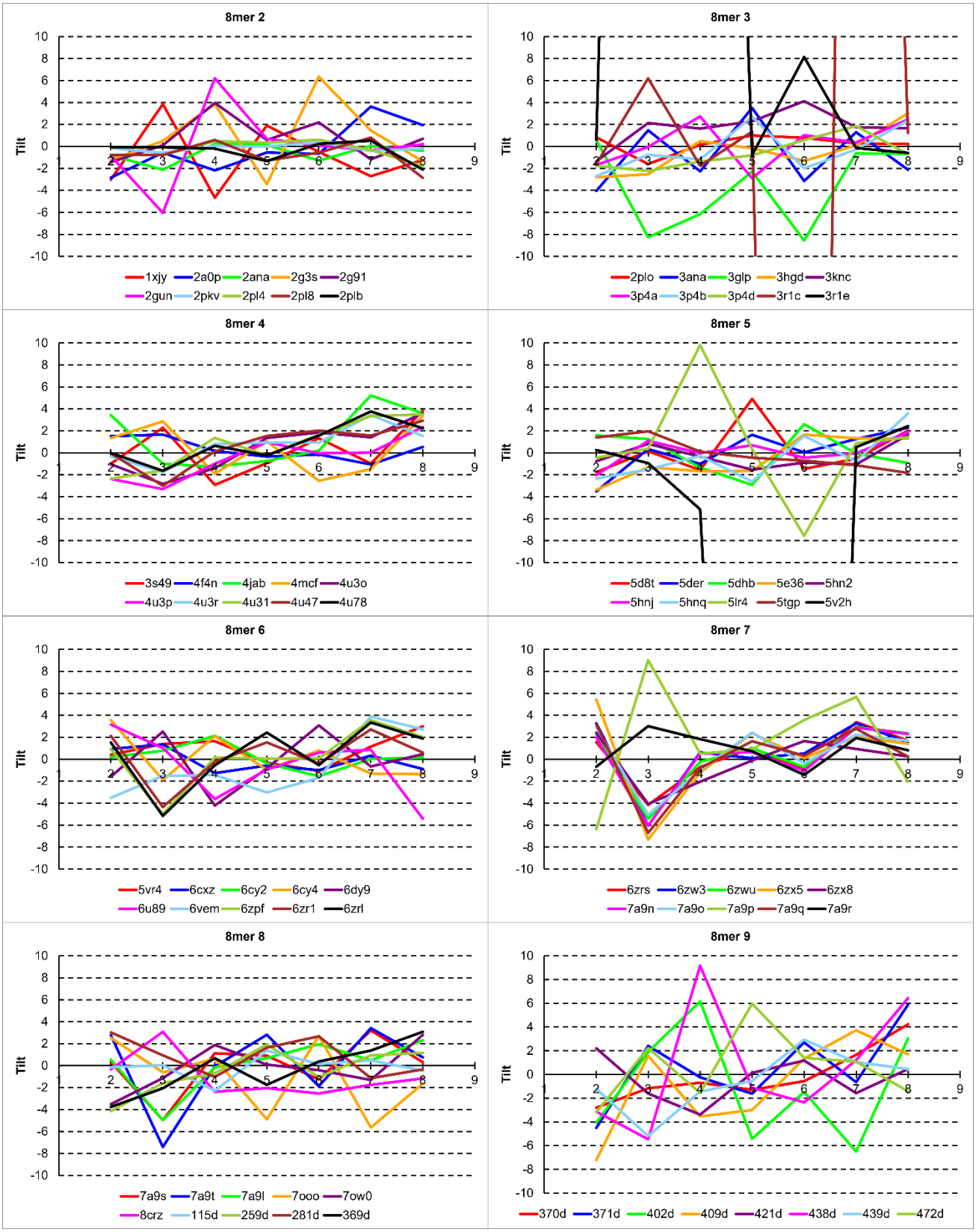
```
            download_link.click()
            source_path = os.path.join(download, 'custom_com.pdb')
            for _ in range(10):
                if os.path.exists(source_path):
                    break
                time.sleep(1)
            base_filename = os.path.splitext(filename)[0].replace("bp_step_", "")
            new_filename = f"3DNABD-{base_filename}.pdb"
            new_file_path = os.path.join(download, new_filename)
            os.rename(source_path, new_file_path)
            print(f"File name was changed to {new_filename}.")
        except Exception as e:
            print(f"An error occurred for file {filename}: {e}")
        finally:
            driver.quit()
print("Processing and model generation finished.")
```

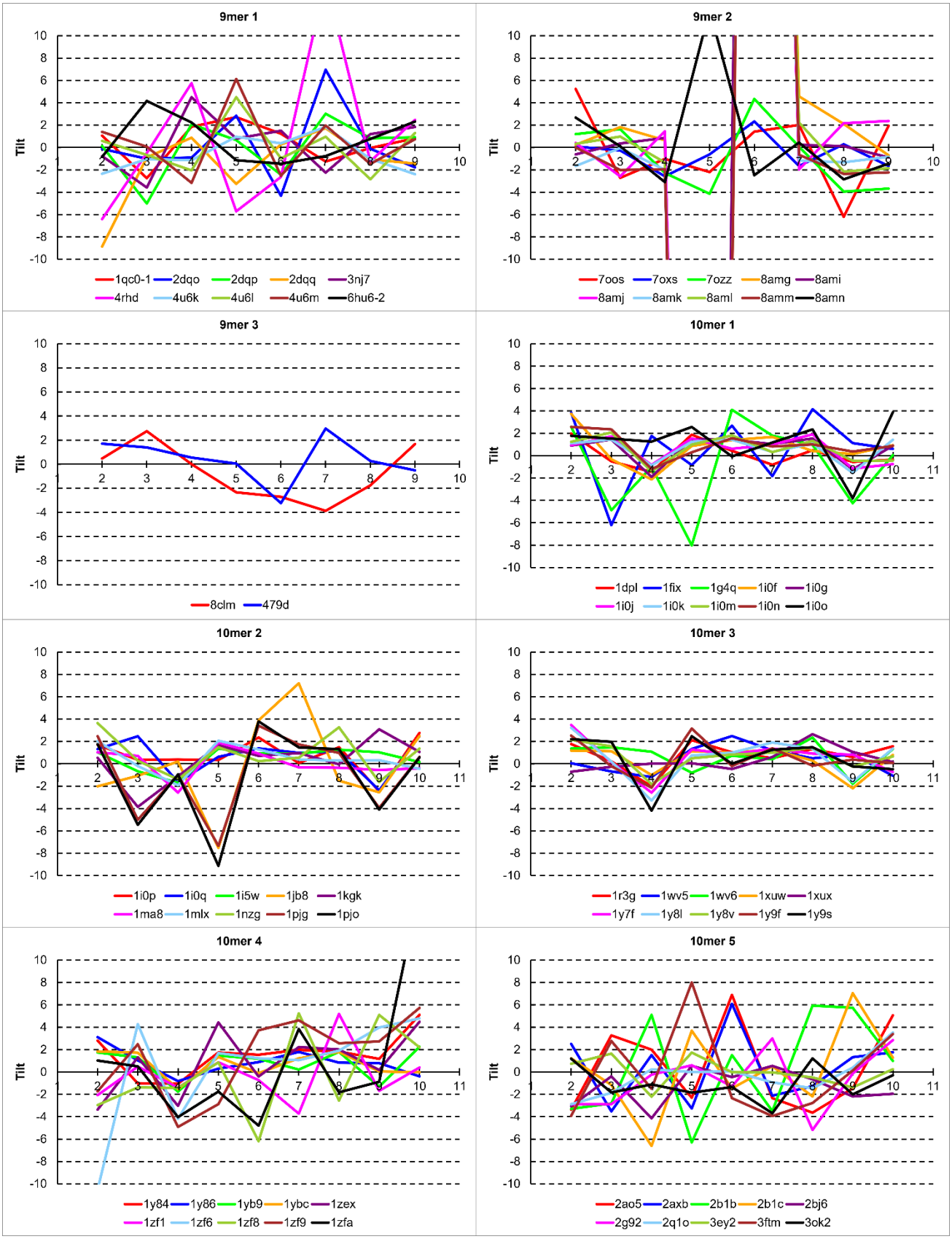
**Script S3:** The shell script for repeatedly performing molecular replacement to investigate the effects of six helical parameters using PDB ID 1IH1.

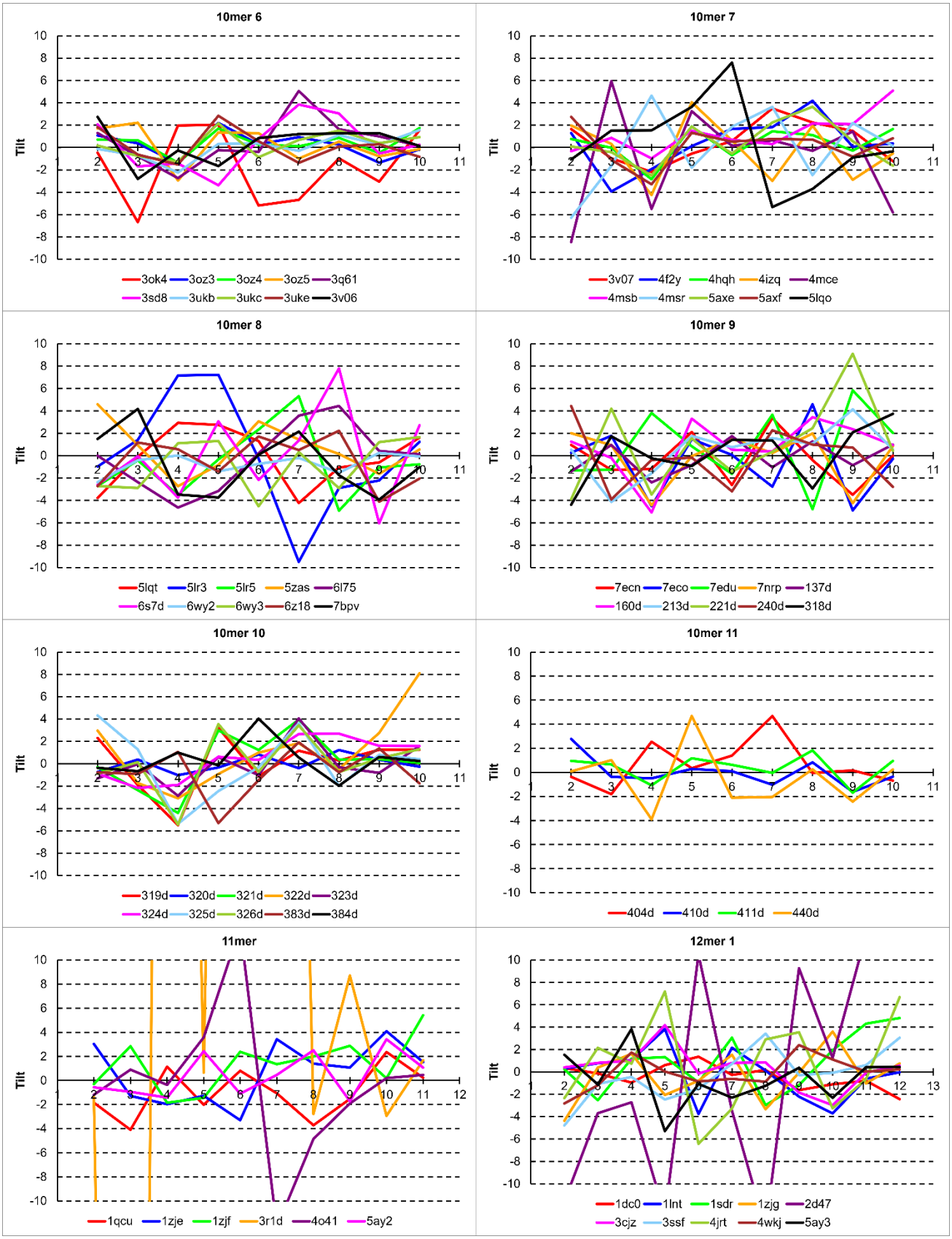
```
pdb_directory=""           # Enter the directory including the diffraction data and models.
cd "${pdb_directory}"
for pdb_file in "$pdb_directory"/*.pdb; do
    output_directory="phaser_$(basename "$pdb_file" .pdb)"
    mkdir -p "$output_directory"
    phaser <<eof
TITLe $(basename "$pdb_file" .pdb)
MODE MR_AUTO
HKLIn reflections.mtz
ENSEmble $(basename "$pdb_file" .pdb) PDB $(basename "$pdb_file" .pdb).pdb IDENtity 80
COMPosition NUCLeic MW 3589 NUM 1
SEARch ENSEmble $(basename "$pdb_file" .pdb) NUM 1
eof
    mv PHASER.sol "$output_directory/"
    mv PHASER.1.mtz "$output_directory/"
    mv PHASER.1.pdb "$output_directory/"
done
```

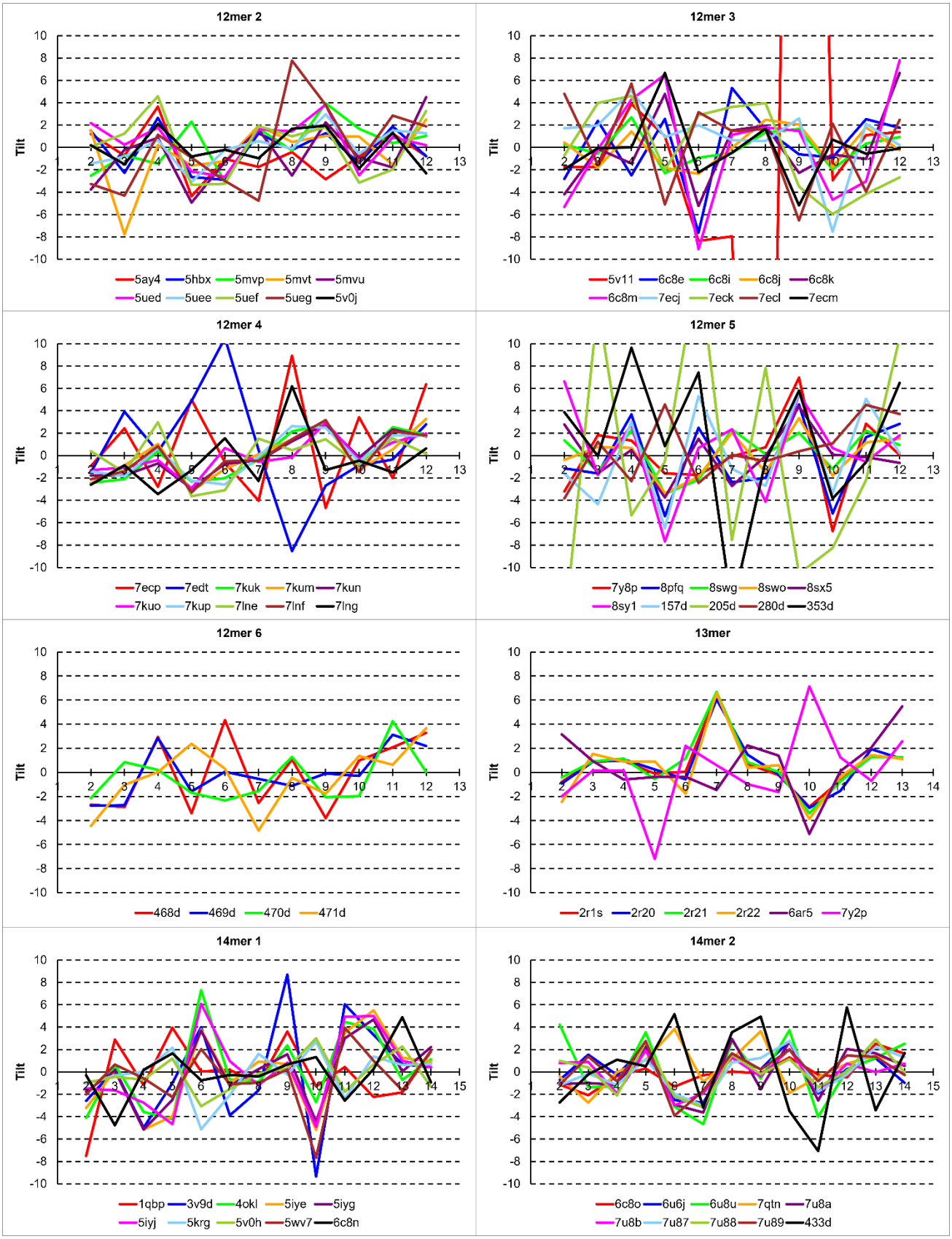
**Figure S1:** The graphs to visualize the regularity of Tilt for the A-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.




8mer 2
Tilt
1xjy 2a0p 2ana 2g3s 2g91
2gun 2pkv 2pl4 2pl8 2plb
8mer 3
Tilt
2plo 3ana 3glp 3hgd 3knc
3p4a 3p4b 3p4d 3r1c 3r1e
8mer 4
Tilt
3s49 4f4n 4jab 4mcf 4u3o
4u3p 4u3r 4u31 4u47 4u78
8mer 5
Tilt
5d8t 5der 5dhb 5e36 5hn2
5hnj 5hnq 5lr4 5tgp 5v2h
8mer 6
Tilt
5vr4 6cxz 6cy2 6cy4 6dy9
6u89 6vem 6zpf 6zr1 6zrl
8mer 7
Tilt
6zrs 6zw3 6zwu 6zx5 6zx8
7a9n 7a9o 7a9p 7a9q 7a9r
8mer 8
Tilt
7a9s 7a9t 7a9l 7ooo 7ow0
8crz 115d 259d 281d 369d
8mer 9
Tilt
370d 371d 402d 409d 421d 438d 439d 472d


9mer 1
Tilt
1qc0-1 2dqo 2dqp 2dqq 3nj7
4rhd 4u6k 4u6l 4u6m 6hu6-2
9mer 2
Tilt
7oos 7oxs 7ozz 8amg 8ami
8amj 8amk 8aml 8amm 8amn
9mer 3
Tilt
8clm 479d
10mer 1
Tilt
1dpl 1fix 1g4q 1i0f 1i0g
1i0j 1i0k 1i0m 1i0n 1i0o
10mer 2
Tilt
1i0p 1i0q 1i5w 1jb8 1kgk
1ma8 1mlx 1nzg 1pjg 1pjo
10mer 3
Tilt
1r3g 1wv5 1wv6 1xuw 1xux
1y7f 1y8l 1y8v 1y9f 1y9s
10mer 4
Tilt
1y84 1y86 1yb9 1ybc 1zex
1zf1 1zf6 1zf8 1zf9 1zfa
10mer 5
Tilt
2ao5 2axb 2b1b 2b1c 2bj6
2g92 2q1o 3ey2 3ftm 3ok2


10mer 6
Tilt
3ok4 3oz3 3oz4 3oz5 3q61
3sd8 3ukb 3ukc 3uke 3v06
10mer 7
Tilt
3v07 4f2y 4hqh 4izq 4mce
4msb 4msr 5axe 5axf 5lqo
10mer 8
Tilt
5lqt 5lr3 5lr5 5zas 6l75
6s7d 6wy2 6wy3 6z18 7bpv
10mer 9
Tilt
7ecn 7eco 7edu 7nrp 137d
160d 213d 221d 240d 318d
10mer 10
Tilt
319d 320d 321d 322d 323d
324d 325d 326d 383d 384d
10mer 11
Tilt
404d 410d 411d 440d
11mer
Tilt
1qcu 1zje 1zjf 3r1d 4o41 5ay2
12mer 1
Tilt
1dc0 1lnt 1sdr 1zjg 2d47
3cjz 3ssf 4jrt 4wkj 5ay3


12mer 2
Tilt
5ay4 5hbx 5mvp 5mvt 5mvu
5ued 5uee 5uef 5ueg 5v0j
12mer 3
Tilt
5v11 6c8e 6c8i 6c8j 6c8k
6c8m 7ecj 7eck 7ecl 7ecm
12mer 4
Tilt
7ecp 7edt 7kuk 7kum 7kun
7kuo 7kup 7lne 7lnf 7lng
12mer 5
Tilt
7y8p 8pfq 8swg 8swo 8sx5
8sy1 157d 205d 280d 353d
12mer 6
Tilt
468d 469d 470d 471d
13mer
Tilt
2r1s 2r20 2r21 2r22 6ar5 7y2p
14mer 1
Tilt
1qbp 3v9d 4okl 5iye 5iyg
5iyj 5krg 5v0h 5wv7 6c8n
14mer 2
Tilt
6c8o 6u6j 6u8u 7qtn 7u8a
7u8b 7u87 7u88 7u89 433d

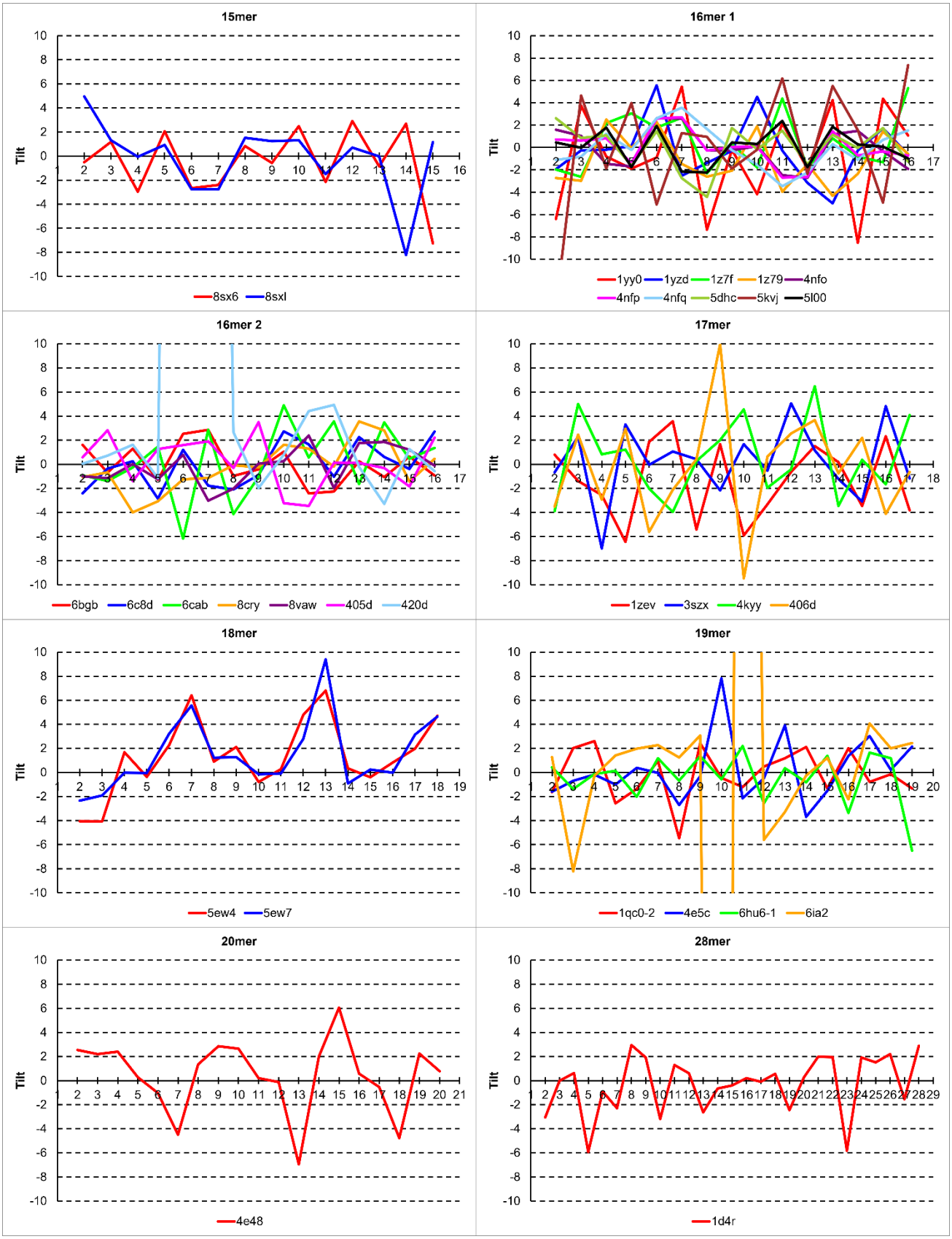
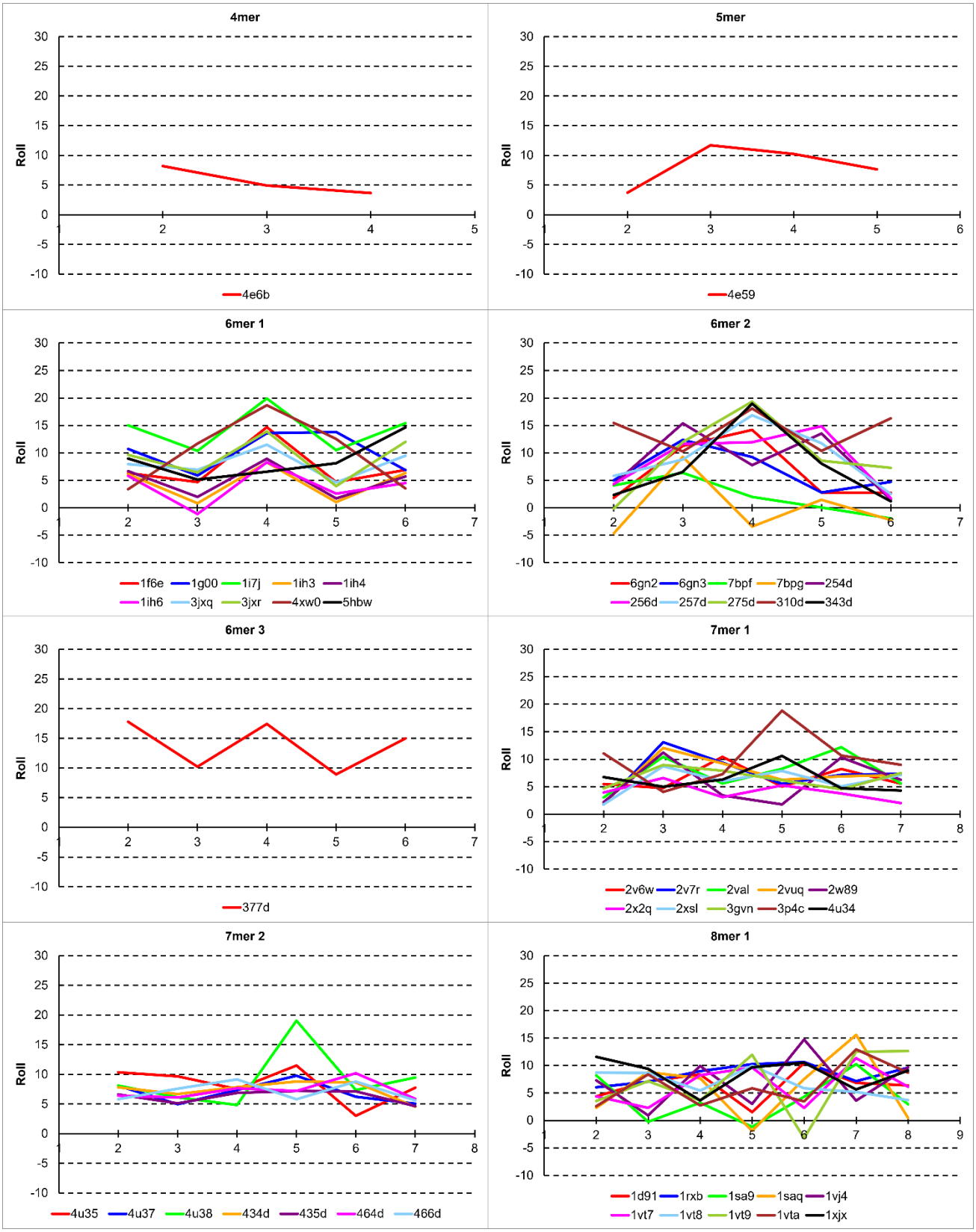
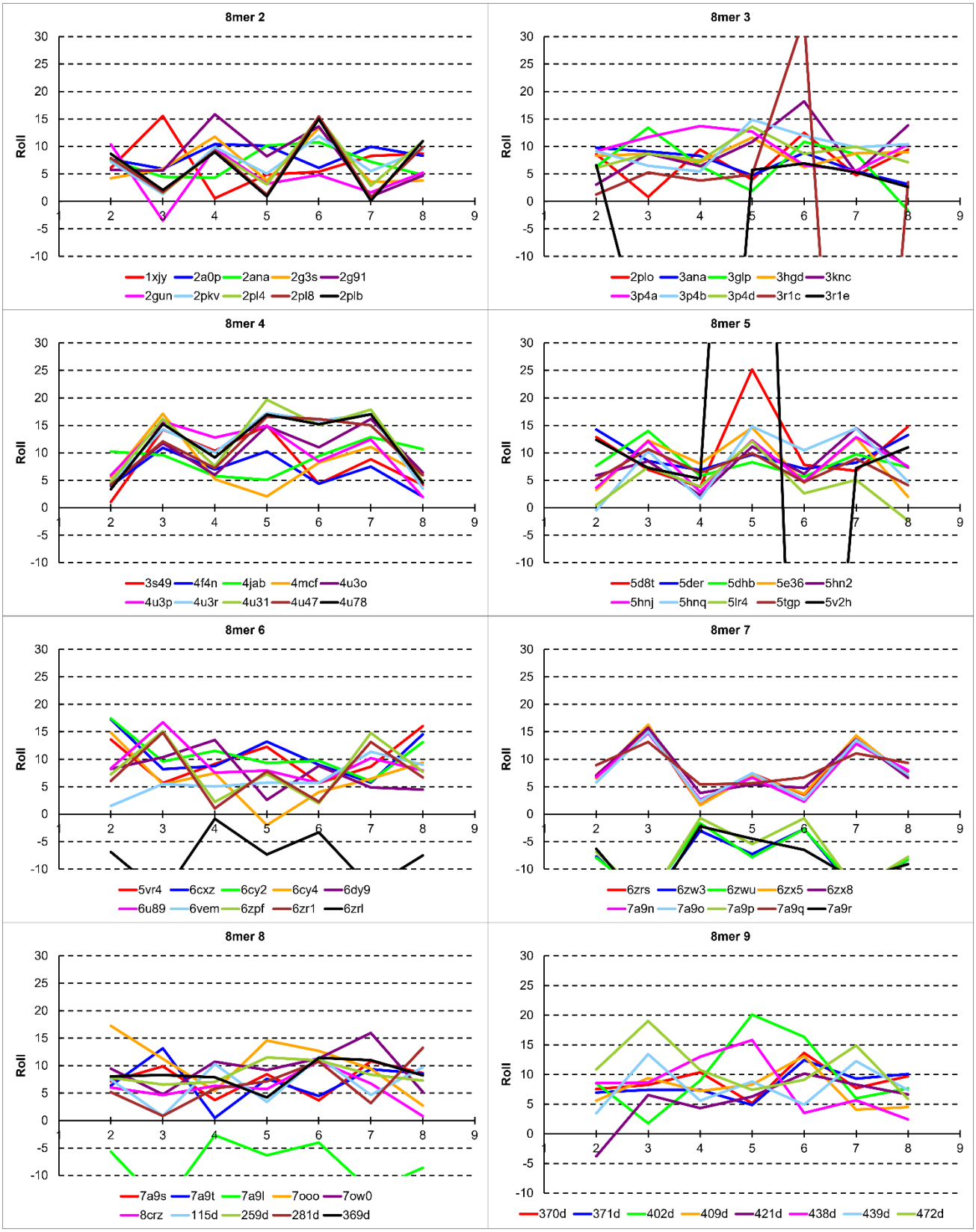
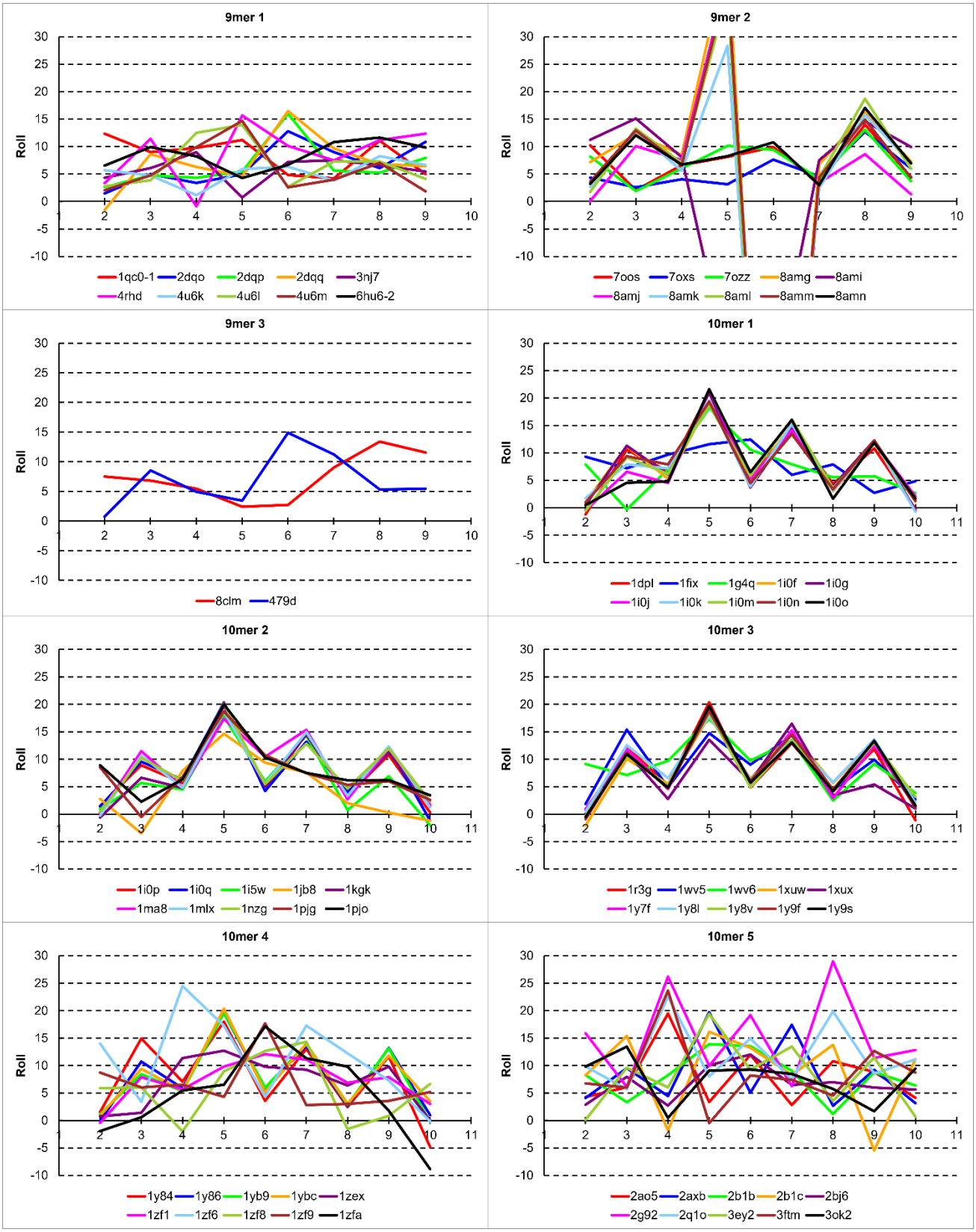
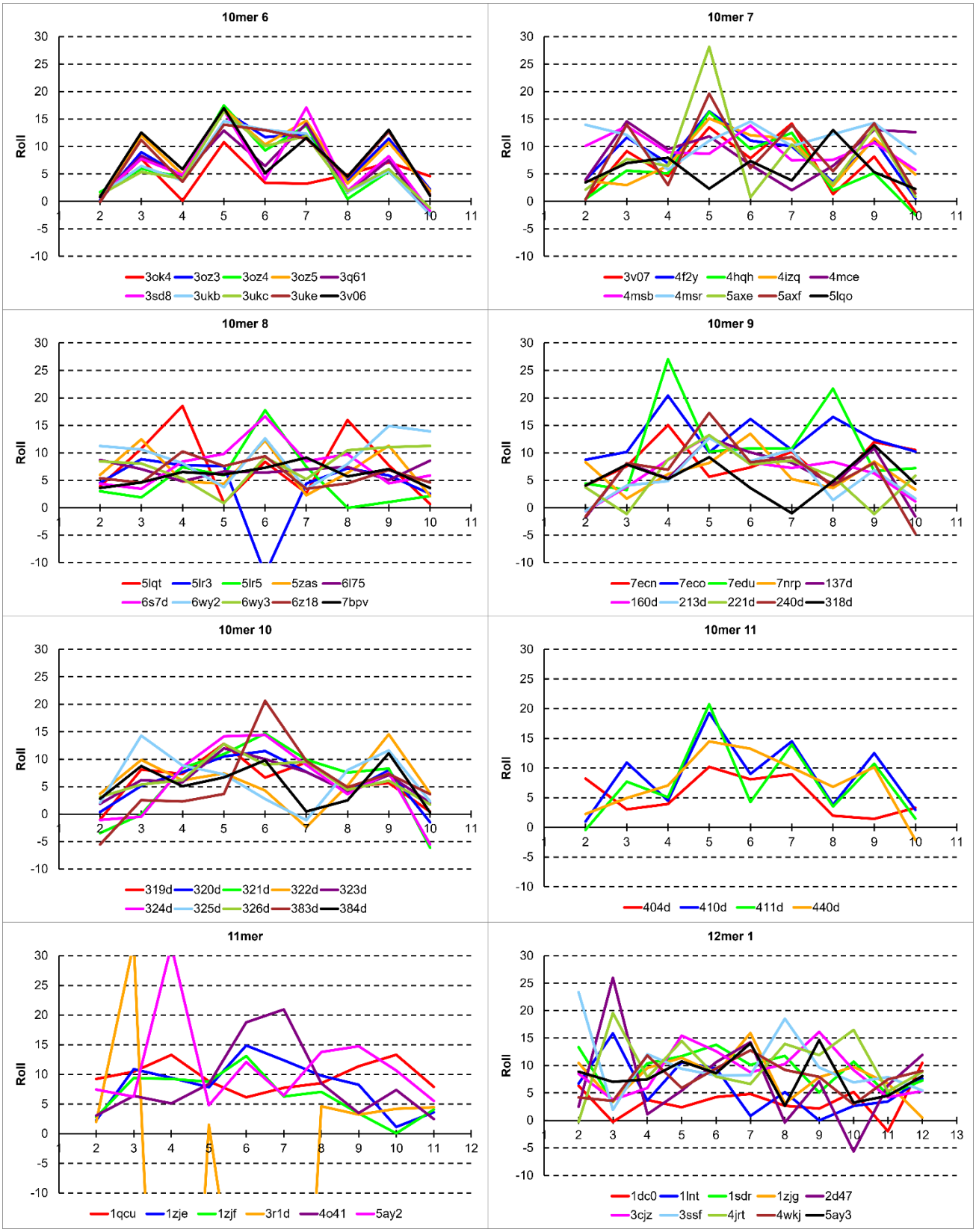

15mer
Tilt
8sx6
8sxl
16mer 1
1yy0
1yzd
1z7f
1z79
4nfo
4nfp
4nfq
5dhc
5kvj
5l00
16mer 2
6bgb
6c8d
6cab
8cry
8vaw
405d
420d
17mer
1zev
3szx
4kyy
406d
18mer
5ew4
5ew7
19mer
1qc0-2
4e5c
6hu6-1
6ia2
20mer
4e48
28mer
1d4r

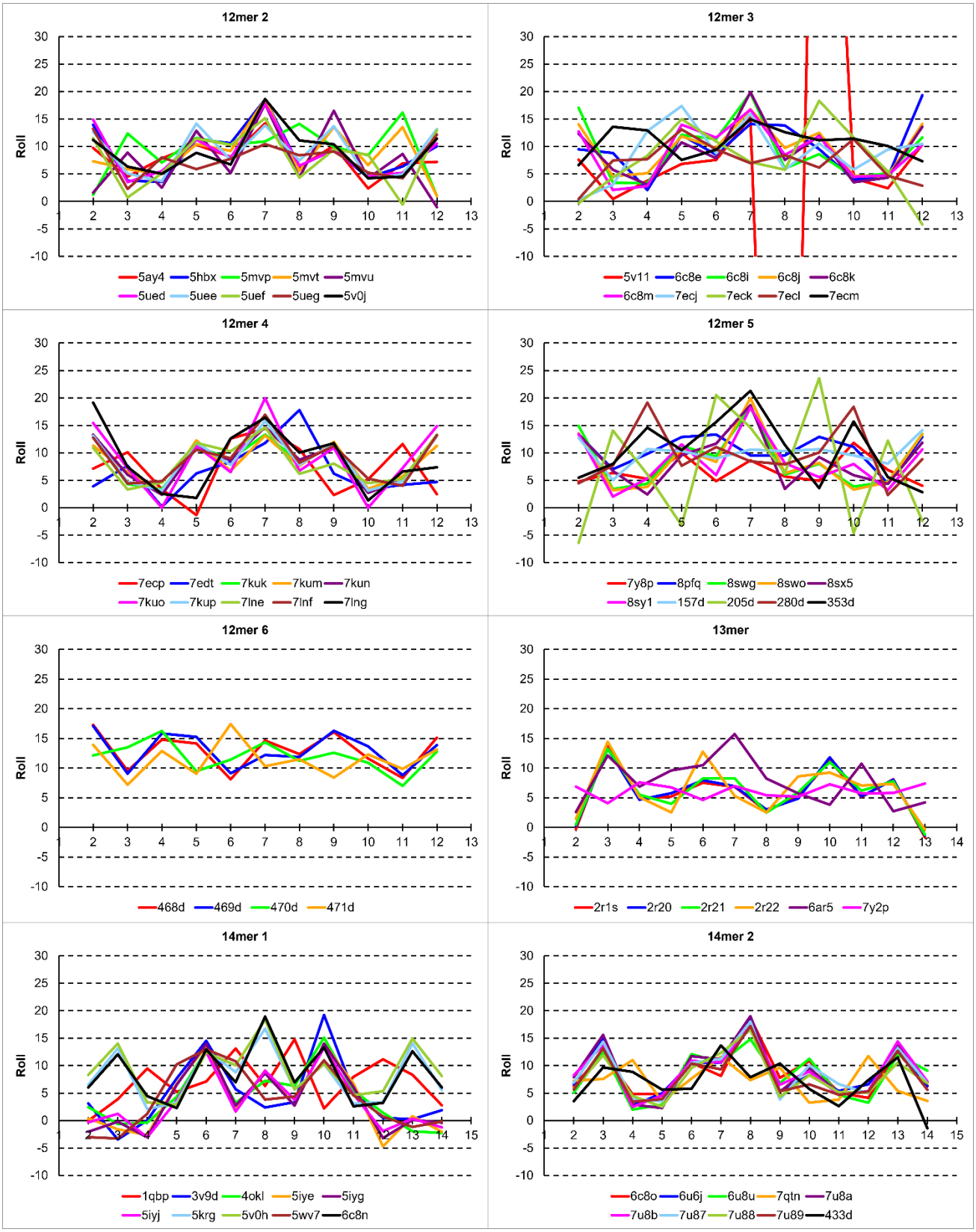
**Figure S2:** The graphs to visualize the regularity of Roll for the A-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.




8mer 2
Roll
1xjy 2a0p 2ana 2g3s 2g91
2gun 2pkv 2pl4 2pl8 2plb
8mer 3
Roll
2plo 3ana 3glp 3hgd 3knc
3p4a 3p4b 3p4d 3r1c 3r1e
8mer 4
Roll
3s49 4f4n 4jab 4mcf 4u3o
4u3p 4u3r 4u31 4u47 4u78
8mer 5
Roll
5d8t 5der 5dhb 5e36 5hn2
5hnj 5hnq 5lr4 5tgp 5v2h
8mer 6
Roll
5vr4 6cxz 6cy2 6cy4 6dy9
6u89 6vem 6zpf 6zr1 6zrl
8mer 7
Roll
6zrs 6zw3 6zwu 6zx5 6zx8
7a9n 7a9o 7a9p 7a9q 7a9r
8mer 8
Roll
7a9s 7a9t 7a9l 7ooo 7ow0
8crz 115d 259d 281d 369d
8mer 9
Roll
370d 371d 402d 409d 421d 438d 439d 472d


9mer 1
Roll
1qc0-1 2dqo 2dqp 2dqq 3nj7
4rhd 4u6k 4u6l 4u6m 6hu6-2
9mer 2
Roll
7oos 7oxs 7ozz 8amg 8ami
8amj 8amk 8aml 8amm 8amn
9mer 3
Roll
8clm 479d
10mer 1
Roll
1dpl 1fix 1g4q 1i0f 1i0g
1i0j 1i0k 1i0m 1i0n 1i0o
10mer 2
Roll
1i0p 1i0q 1i5w 1jb8 1kgk
1ma8 1mlx 1nzg 1pjg 1pjo
10mer 3
Roll
1r3g 1wv5 1wv6 1xuw 1xux
1y7f 1y8l 1y8v 1y9f 1y9s
10mer 4
Roll
1y84 1y86 1yb9 1ybc 1zex
1zf1 1zf6 1zf8 1zf9 1zfa
10mer 5
Roll
2ao5 2axb 2b1b 2b1c 2bj6
2g92 2q1o 3ey2 3ftm 3ok2


10mer 6
Roll
3ok4 3oz3 3oz4 3oz5 3q61
3sd8 3ukb 3ukc 3uke 3v06
10mer 7
Roll
3v07 4f2y 4hqh 4izq 4mce
4msb 4msr 5axe 5axf 5lqo
10mer 8
Roll
5lqt 5lr3 5lr5 5zas 6l75
6s7d 6wy2 6wy3 6z18 7bpv
10mer 9
Roll
7ecn 7eco 7edu 7nrp 137d
160d 213d 221d 240d 318d
10mer 10
Roll
319d 320d 321d 322d 323d
324d 325d 326d 383d 384d
10mer 11
Roll
404d 410d 411d 440d
11mer
Roll
1qcu 1zje 1zjf 3r1d 4o41 5ay2
12mer 1
Roll
1dc0 1lnt 1sdr 1zjg 2d47
3cjz 3ssf 4jrt 4wkj 5ay3


12mer 2
Roll
5ay4 5hbx 5mvp 5mvt 5mvu
5ued 5uee 5uef 5ueg 5v0j
12mer 3
Roll
5v11 6c8e 6c8i 6c8j 6c8k
6c8m 7ecj 7eck 7ecl 7ecm
12mer 4
Roll
7ecp 7edt 7kuk 7kum 7kun
7kuo 7kup 7lne 7lnf 7lng
12mer 5
Roll
7y8p 8pfq 8swg 8swo 8sx5
8sy1 157d 205d 280d 353d
12mer 6
Roll
468d 469d 470d 471d
13mer
Roll
2r1s 2r20 2r21 2r22 6ar5 7y2p
14mer 1
Roll
1qbp 3v9d 4okl 5iye 5iyg
5iyj 5krg 5v0h 5wv7 6c8n
14mer 2
Roll
6c8o 6u6j 6u8u 7qtn 7u8a
7u8b 7u87 7u88 7u89 433d

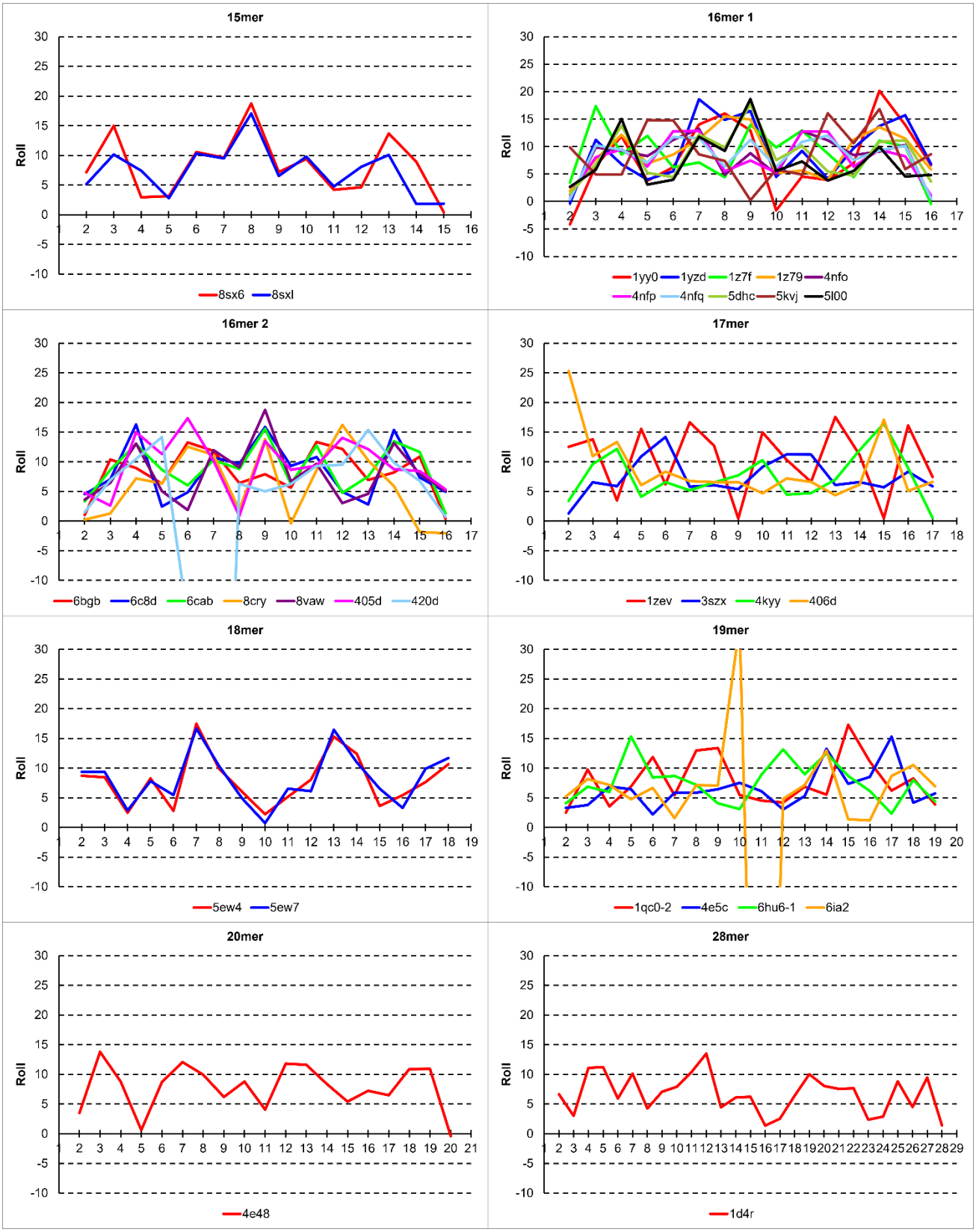
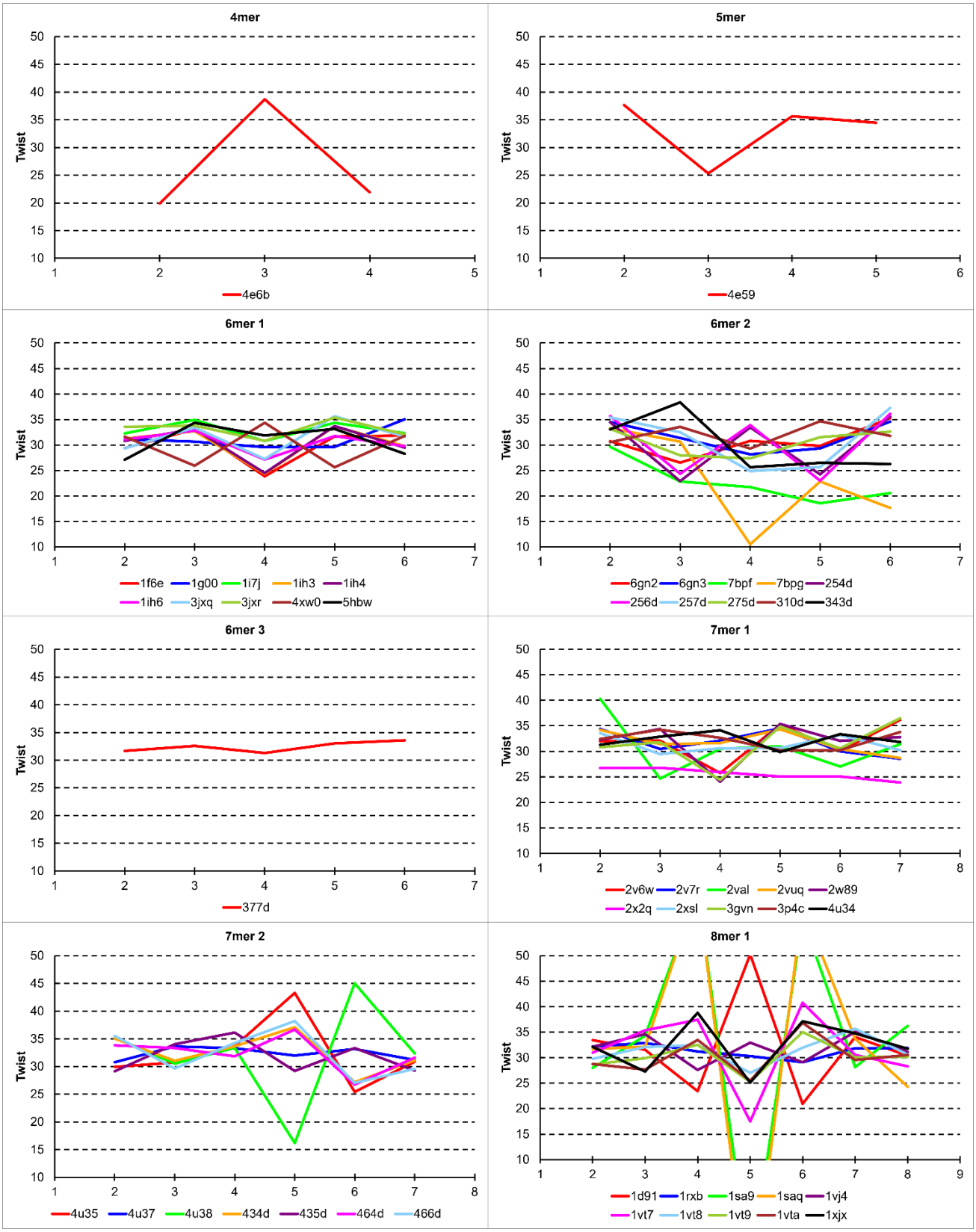
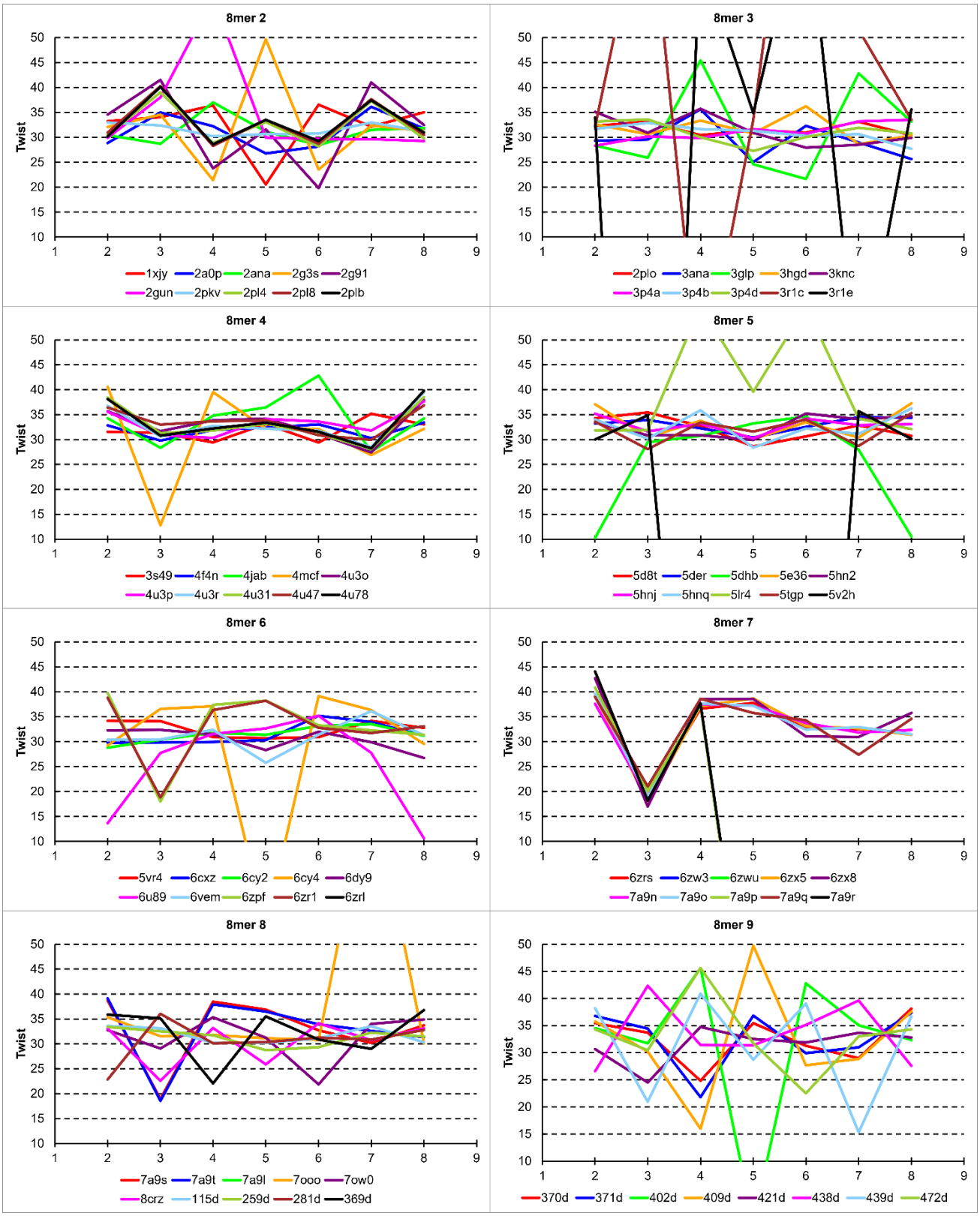
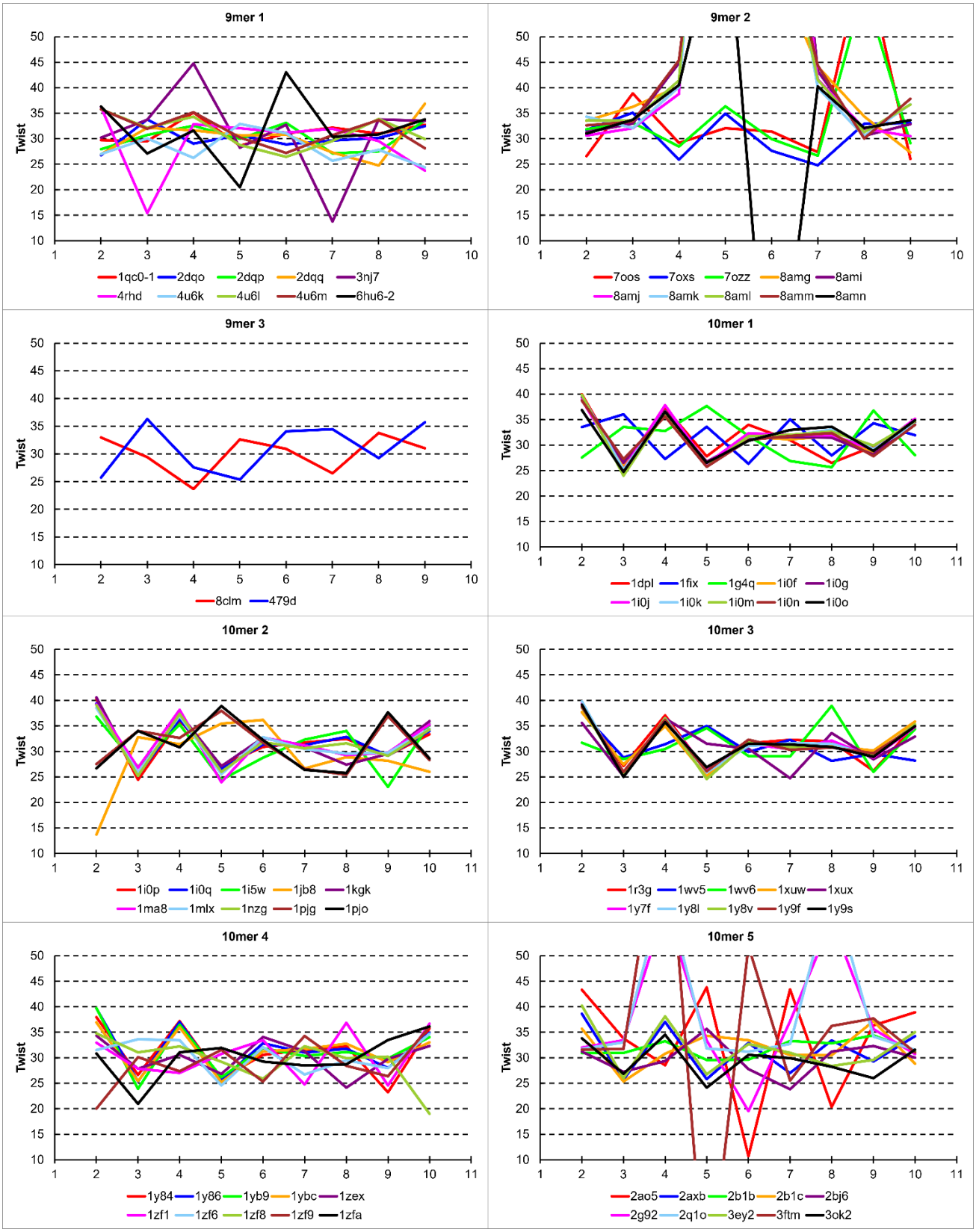
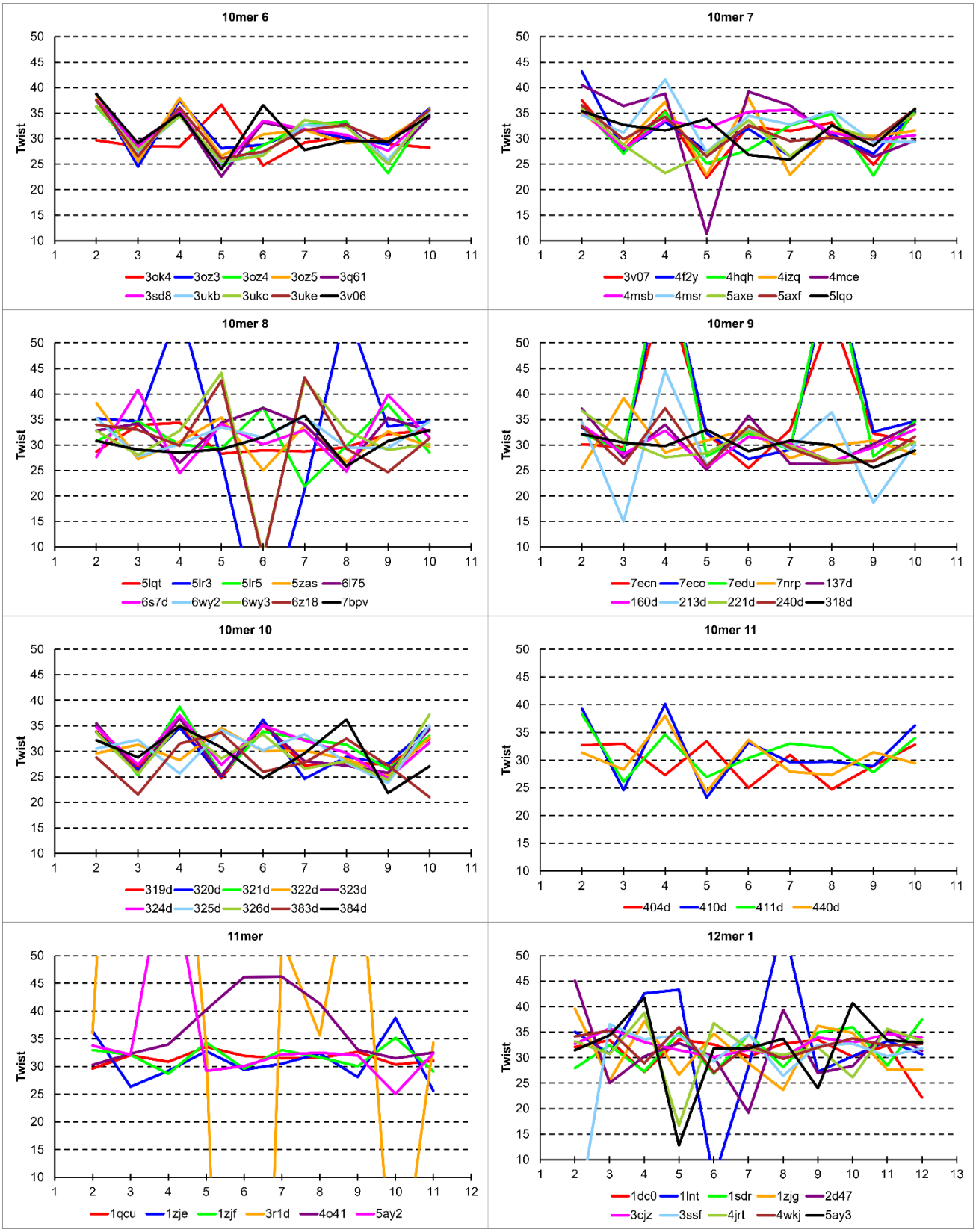

15mer
Roll
8sx6 8sxl
16mer 1
1yy0 1yzd 1z7f 1z79 4nfo
4nfp 4nfq 5dhc 5kvj 5l00
16mer 2
6bgb 6c8d 6cab 8cry 8vaw 405d 420d
17mer
1zev 3szx 4kyy 406d
18mer
5ew4 5ew7
19mer
1qc0-2 4e5c 6hu6-1 6ia2
20mer
4e48
28mer
1d4r

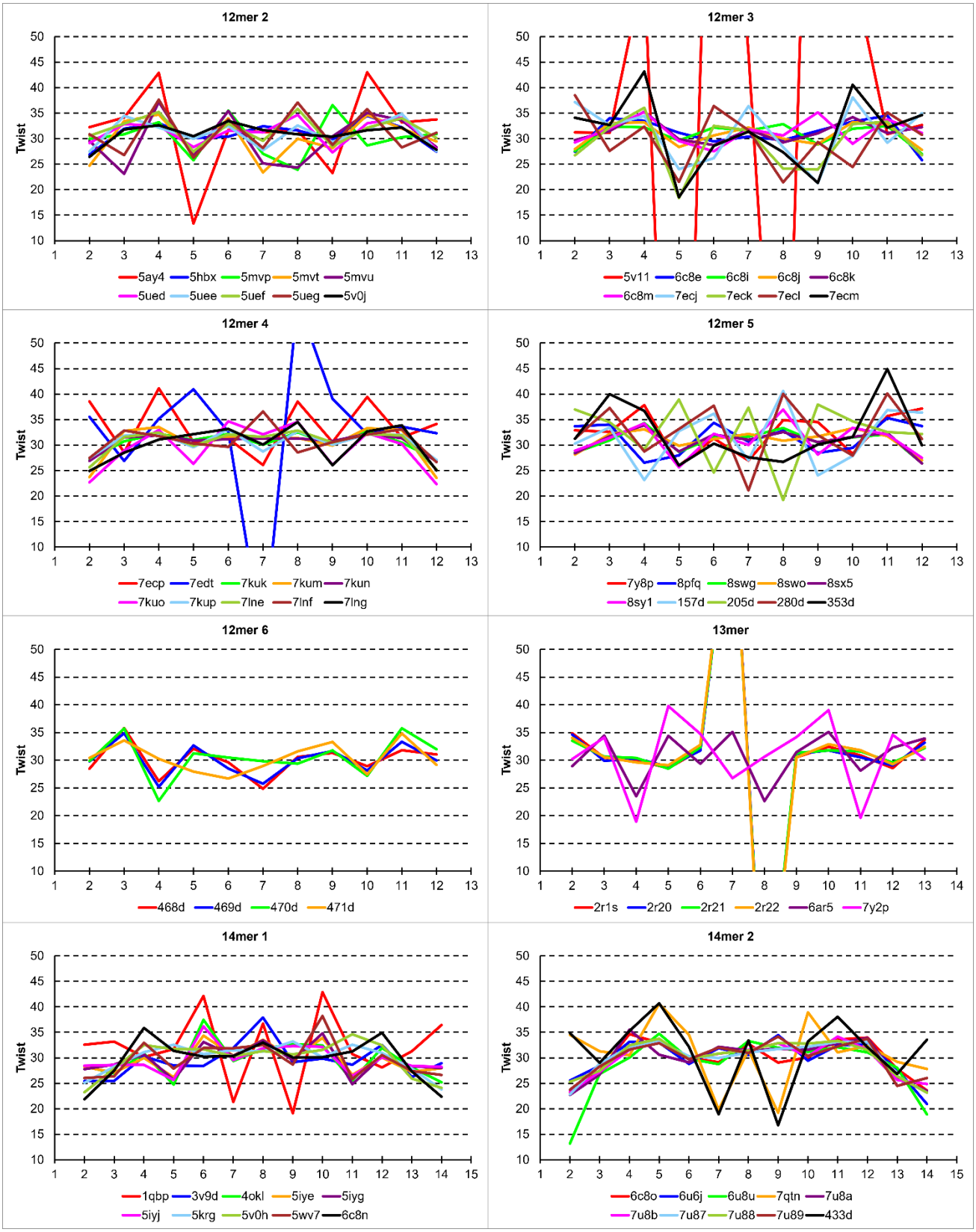
**Figure S3:** The graphs to visualize the regularity of Twist for the A-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.




8mer 2
Twist
1xjy 2a0p 2ana 2g3s 2g91
2gun 2pkv 2pl4 2pl8 2plb
8mer 3
Twist
2plo 3ana 3glp 3hgd 3knc
3p4a 3p4b 3p4d 3r1c 3r1e
8mer 4
Twist
3s49 4f4n 4jab 4mcf 4u3o
4u3p 4u3r 4u31 4u47 4u78
8mer 5
Twist
5d8t 5der 5dhb 5e36 5hn2
5hnj 5hnq 5lr4 5tgp 5v2h
8mer 6
Twist
5vr4 6cxz 6cy2 6cy4 6dy9
6u89 6vem 6zpf 6zr1 6zrl
8mer 7
Twist
6zrs 6zw3 6zwu 6zx5 6zx8
7a9n 7a9o 7a9p 7a9q 7a9r
8mer 8
Twist
7a9s 7a9t 7a9l 7ooo 7ow0
8crz 115d 259d 281d 369d
8mer 9
Twist
370d 371d 402d 409d 421d 438d 439d 472d


9mer 1
Twist
1qc0-1 2dqo 2dqp 2dqq 3nj7
4rhd 4u6k 4u6l 4u6m 6hu6-2
9mer 2
Twist
7oos 7oxs 7ozz 8amg 8ami
8amj 8amk 8aml 8amm 8amn
9mer 3
Twist
8clm 479d
10mer 1
Twist
1dpl 1fix 1g4q 1i0f 1i0g
1i0j 1i0k 1i0m 1i0n 1i0o
10mer 2
Twist
1i0p 1i0q 1i5w 1jb8 1kgk
1ma8 1mlx 1nzg 1pjg 1pjo
10mer 3
Twist
1r3g 1wv5 1wv6 1xuw 1xux
1y7f 1y8l 1y8v 1y9f 1y9s
10mer 4
Twist
1y84 1y86 1yb9 1ybc 1zex
1zf1 1zf6 1zf8 1zf9 1zfa
10mer 5
Twist
2ao5 2axb 2b1b 2b1c 2bj6
2g92 2q1o 3ey2 3ftm 3ok2


10mer 6
Twist
3ok4 3oz3 3oz4 3oz5 3q61
3sd8 3ukb 3ukc 3uke 3v06
10mer 7
Twist
3v07 4f2y 4hqh 4izq 4mce
4msb 4msr 5axe 5axf 5lqo
10mer 8
Twist
5lqt 5lr3 5lr5 5zas 6l75
6s7d 6wy2 6wy3 6z18 7bpv
10mer 9
Twist
7ecn 7eco 7edu 7nrp 137d
160d 213d 221d 240d 318d
10mer 10
Twist
319d 320d 321d 322d 323d
324d 325d 326d 383d 384d
10mer 11
Twist
404d 410d 411d 440d
11mer
Twist
1qcu 1zje 1zjf 3r1d 4o41 5ay2
12mer 1
Twist
1dc0 1lnt 1sdr 1zjg 2d47
3cjz 3ssf 4jrt 4wkj 5ay3


12mer 2
Twist
5ay4 5hbx 5mvp 5mvt 5mvu
5ued 5uee 5uef 5ueg 5v0j
12mer 3
Twist
5v11 6c8e 6c8i 6c8j 6c8k
6c8m 7ecj 7eck 7ecl 7ecm
12mer 4
Twist
7ecp 7edt 7kuk 7kum 7kun
7kuo 7kup 7lne 7lnf 7lng
12mer 5
Twist
7y8p 8pfq 8swg 8swo 8sx5
8sy1 157d 205d 280d 353d
12mer 6
Twist
468d 469d 470d 471d
13mer
Twist
2r1s 2r20 2r21 2r22 6ar5 7y2p
14mer 1
Twist
1qbp 3v9d 4okl 5iye 5iyg
5iyj 5krg 5v0h 5wv7 6c8n
14mer 2
Twist
6c8o 6u6j 6u8u 7qtn 7u8a
7u8b 7u87 7u88 7u89 433d

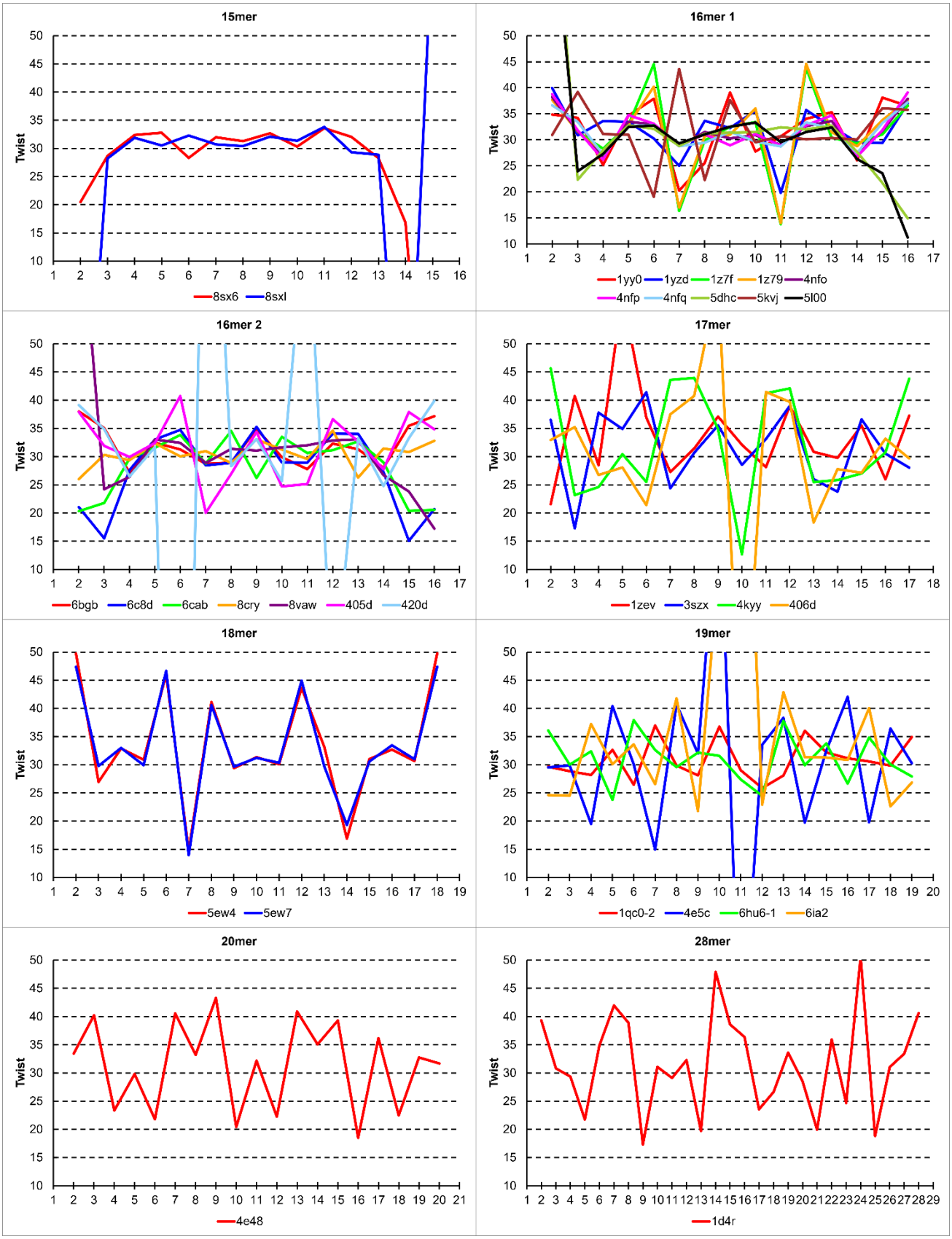
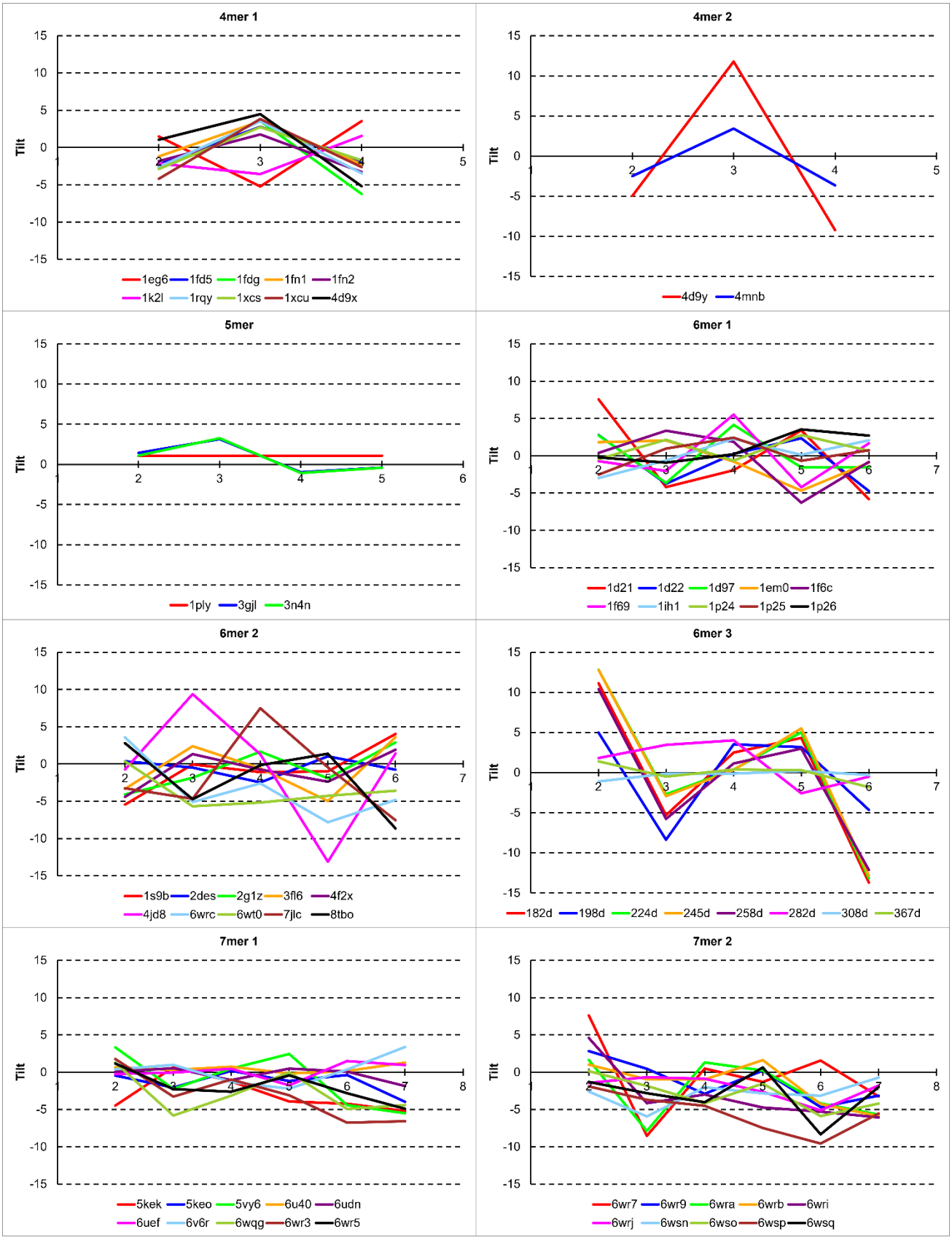
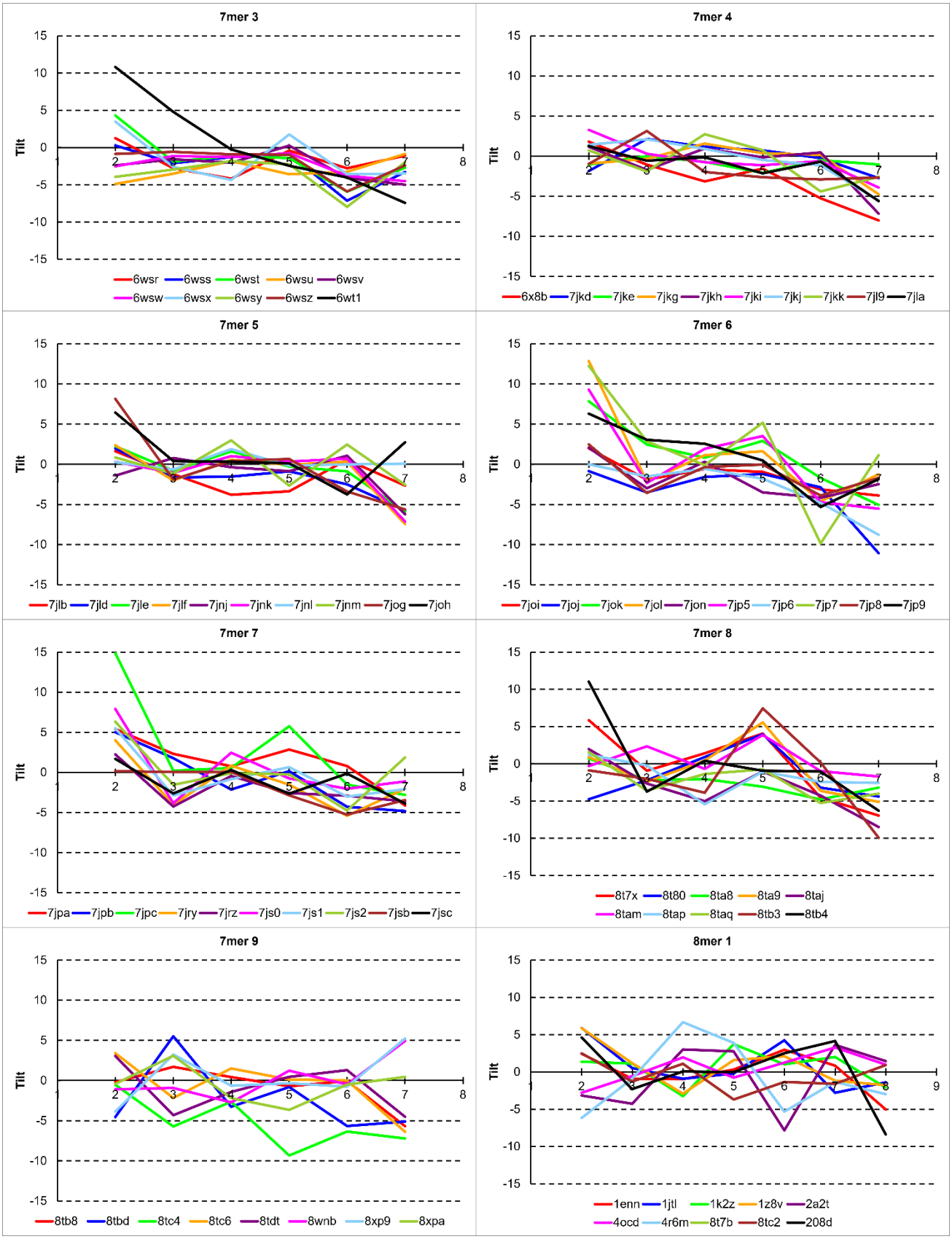
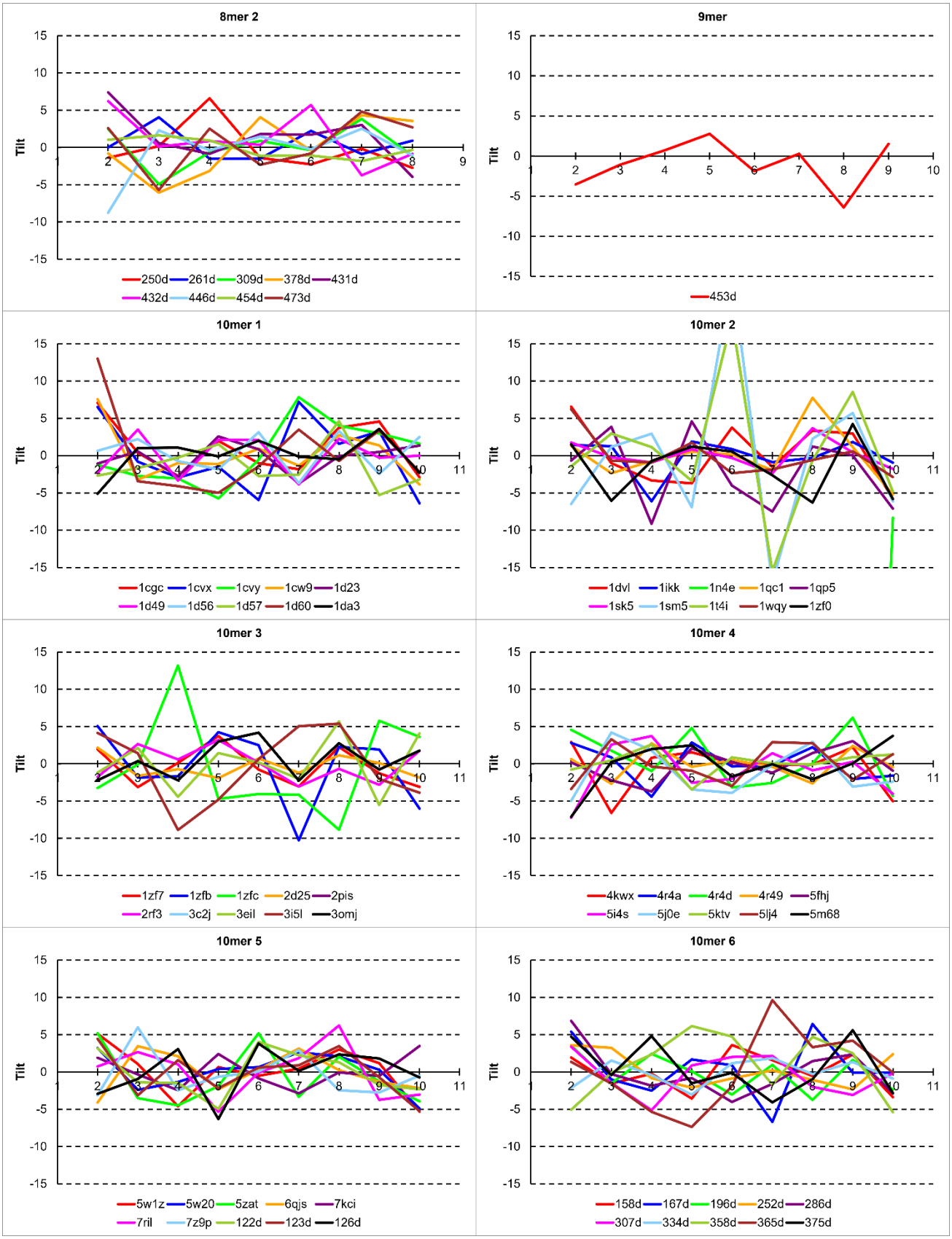
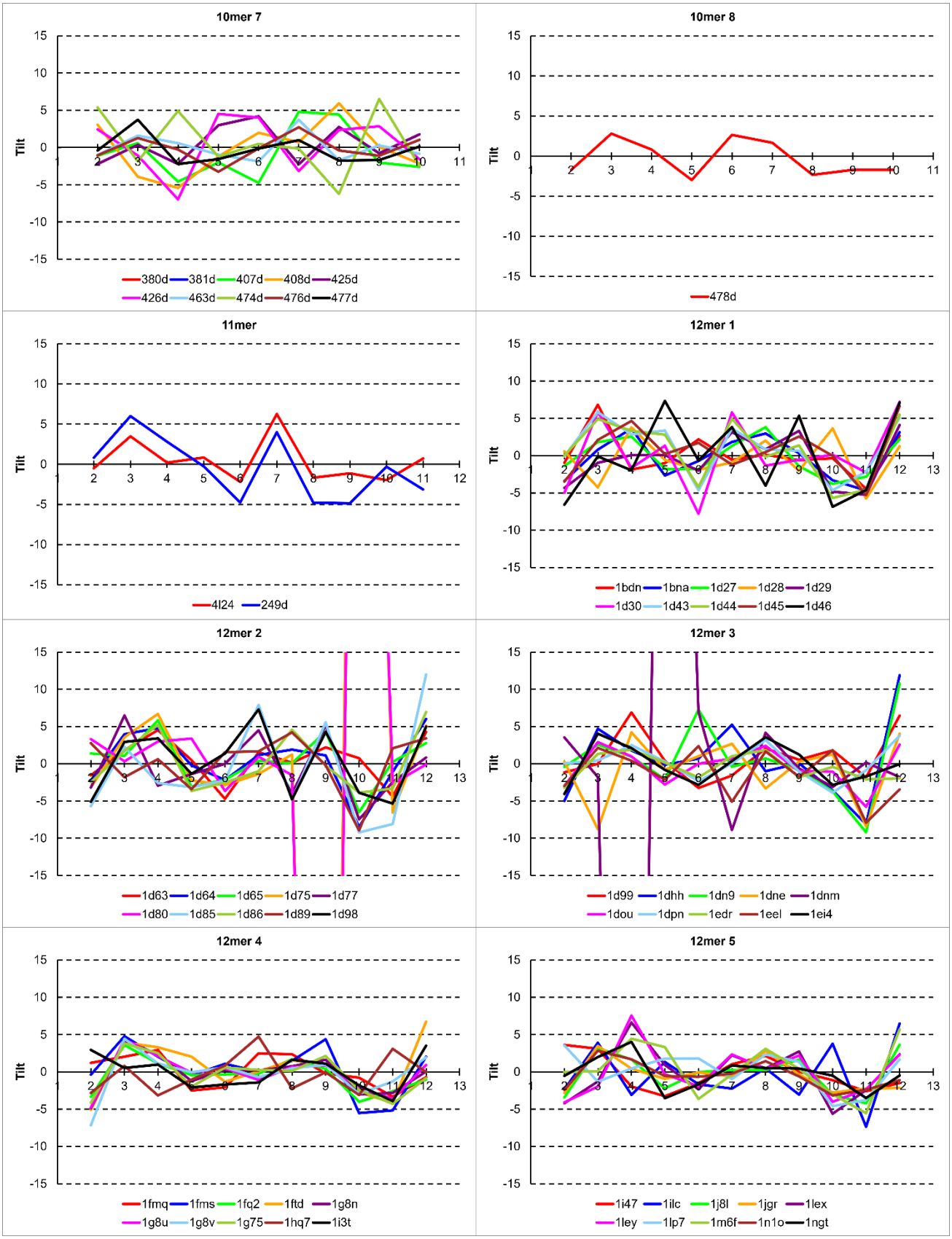

15mer
Twist
8sx6
8sxl
16mer 1
1yy0
1yzd
1z7f
1z79
4nfo
4nfp
4nfq
5dhc
5kvj
5l00
16mer 2
6bgb
6c8d
6cab
8cry
8vaw
405d
420d
17mer
1zev
3szx
4kyy
406d
18mer
5ew4
5ew7
19mer
1qc0-2
4e5c
6hu6-1
6ia2
20mer
4e48
28mer
1d4r

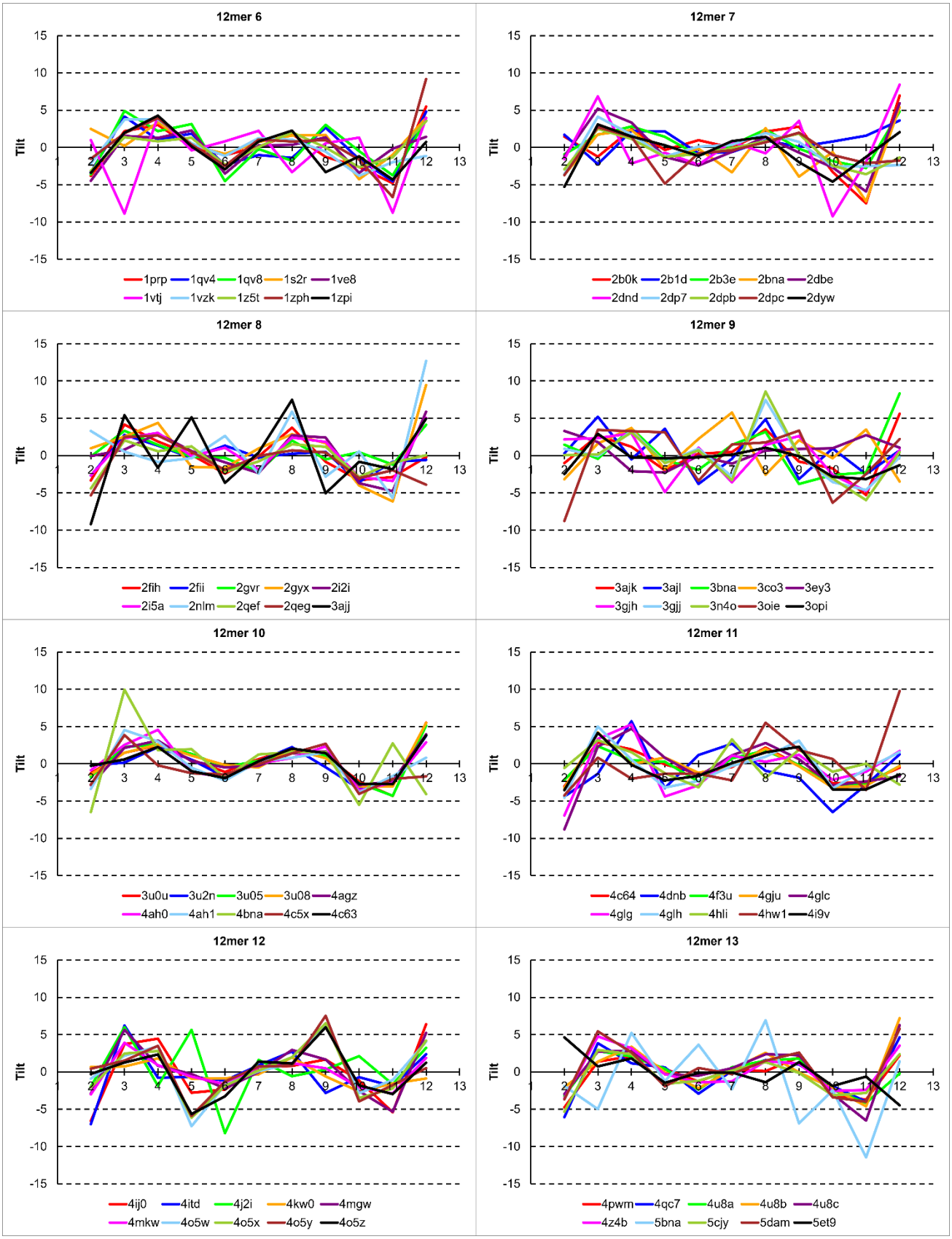
**Figure S4:** The graphs to visualize the regularity of Tilt for the B-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.

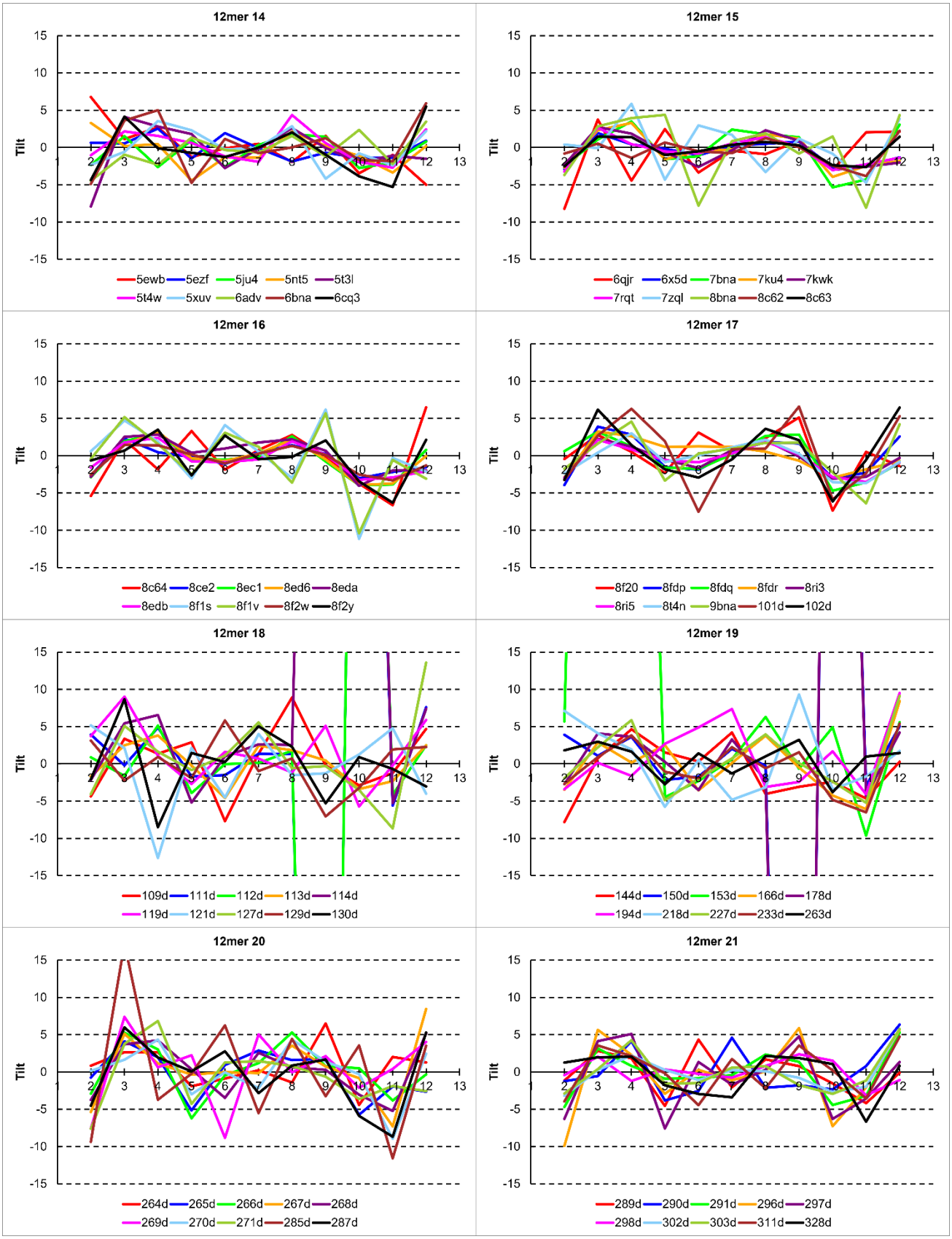



7mer 3
Tilt
6wsr 6wss 6wst 6wsu 6wsv
6wsw 6wsx 6wsy 6wsz 6wt1
7mer 4
Tilt
6x8b 7jkd 7jke 7jkg 7jkh 7jki 7jkj 7jkk 7jl9 7jla
7mer 5
Tilt
7jlb 7jld 7jle 7jlf 7jnj 7jnk 7jnl 7jnm 7jog 7joh
7mer 6
Tilt
7joi 7joj 7jok 7jol 7jon 7jp5 7jp6 7jp7 7jp8 7jp9
7mer 7
Tilt
7jpa 7jpb 7jpc 7jry 7jrz 7js0 7js1 7js2 7jsb 7jsc
7mer 8
Tilt
8t7x 8t80 8ta8 8ta9 8taj
8tam 8tap 8taq 8tb3 8tb4
7mer 9
Tilt
8tb8 8tbd 8tc4 8tc6 8tdt 8wnb 8xp9 8xpa
8mer 1
Tilt
1enn 1jtl 1k2z 1z8v 2a2t
4ocd 4r6m 8t7b 8tc2 208d


8mer 2
Tilt
250d 261d 309d 378d 431d
432d 446d 454d 473d
9mer
Tilt
453d
10mer 1
Tilt
1cgc 1cvx 1cvy 1cw9 1d23
1d49 1d56 1d57 1d60 1da3
10mer 2
Tilt
1dvl 1ikk 1n4e 1qc1 1qp5
1sk5 1sm5 1t4i 1wqy 1zf0
10mer 3
Tilt
1zf7 1zfb 1zfc 2d25 2pis
2rf3 3c2j 3eil 3i5l 3omj
10mer 4
Tilt
4kwx 4r4a 4r4d 4r49 5fhj
5i4s 5j0e 5ktv 5lj4 5m68
10mer 5
Tilt
5w1z 5w20 5zat 6qjs 7kci
7ril 7z9p 122d 123d 126d
10mer 6
Tilt
158d 167d 196d 252d 286d
307d 334d 358d 365d 375d


10mer 7
Tilt
380d 381d 407d 408d 425d
426d 463d 474d 476d 477d
10mer 8
Tilt
478d
11mer
Tilt
4l24 249d
12mer 1
Tilt
1bdn 1bna 1d27 1d28 1d29
1d30 1d43 1d44 1d45 1d46
12mer 2
Tilt
1d63 1d64 1d65 1d75 1d77
1d80 1d85 1d86 1d89 1d98
12mer 3
Tilt
1d99 1dhh 1dn9 1dne 1dnm
1dou 1dpn 1edr 1eel 1ei4
12mer 4
Tilt
1fmq 1fms 1fq2 1ftd 1g8n
1g8u 1g8v 1g75 1hq7 1i3t
12mer 5
Tilt
1i47 1ilc 1j8l 1jgr 1lex
1ley 1lp7 1m6f 1n1o 1ngt


12mer 6
Tilt
1prp 1qv4 1qv8 1s2r 1ve8
1vtj 1vzk 1z5t 1zph 1zpi
12mer 7
Tilt
2b0k 2b1d 2b3e 2bna 2dbe
2dnd 2dp7 2dpb 2dpc 2dyw
12mer 8
Tilt
2fih 2fii 2gvr 2gyx 2i2i
2i5a 2nlm 2qef 2qeg 3ajj
12mer 9
Tilt
3ajk 3ajl 3bna 3co3 3ey3
3gjh 3gjj 3n4o 3oie 3opi
12mer 10
Tilt
3u0u 3u2n 3u05 3u08 4agz
4ah0 4ah1 4bna 4c5x 4c63
12mer 11
Tilt
4c64 4dnb 4f3u 4gju 4glc
4glg 4glh 4hli 4hw1 4i9v
12mer 12
Tilt
4ij0 4itd 4j2i 4kw0 4mgw
4mkw 4o5w 4o5x 4o5y 4o5z
12mer 13
Tilt
4pwm 4qc7 4u8a 4u8b 4u8c
4z4b 5bna 5cjy 5dam 5et9


12mer 14
Tilt
5ewb 5ezf 5ju4 5nt5 5t3l
5t4w 5xuv 6adv 6bna 6cq3
12mer 15
Tilt
6qjr 6x5d 7bna 7ku4 7kwk
7rqt 7zql 8bna 8c62 8c63
12mer 16
Tilt
8c64 8ce2 8ec1 8ed6 8eda
8edb 8f1s 8f1v 8f2w 8f2y
12mer 17
Tilt
8f20 8fdp 8fdq 8fdr 8ri3
8ri5 8t4n 9bna 101d 102d
12mer 18
Tilt
109d 111d 112d 113d 114d
119d 121d 127d 129d 130d
12mer 19
Tilt
144d 150d 153d 166d 178d
194d 218d 227d 233d 263d
12mer 20
Tilt
264d 265d 266d 267d 268d
269d 270d 271d 285d 287d
12mer 21
Tilt
289d 290d 291d 296d 297d
298d 302d 303d 311d 328d

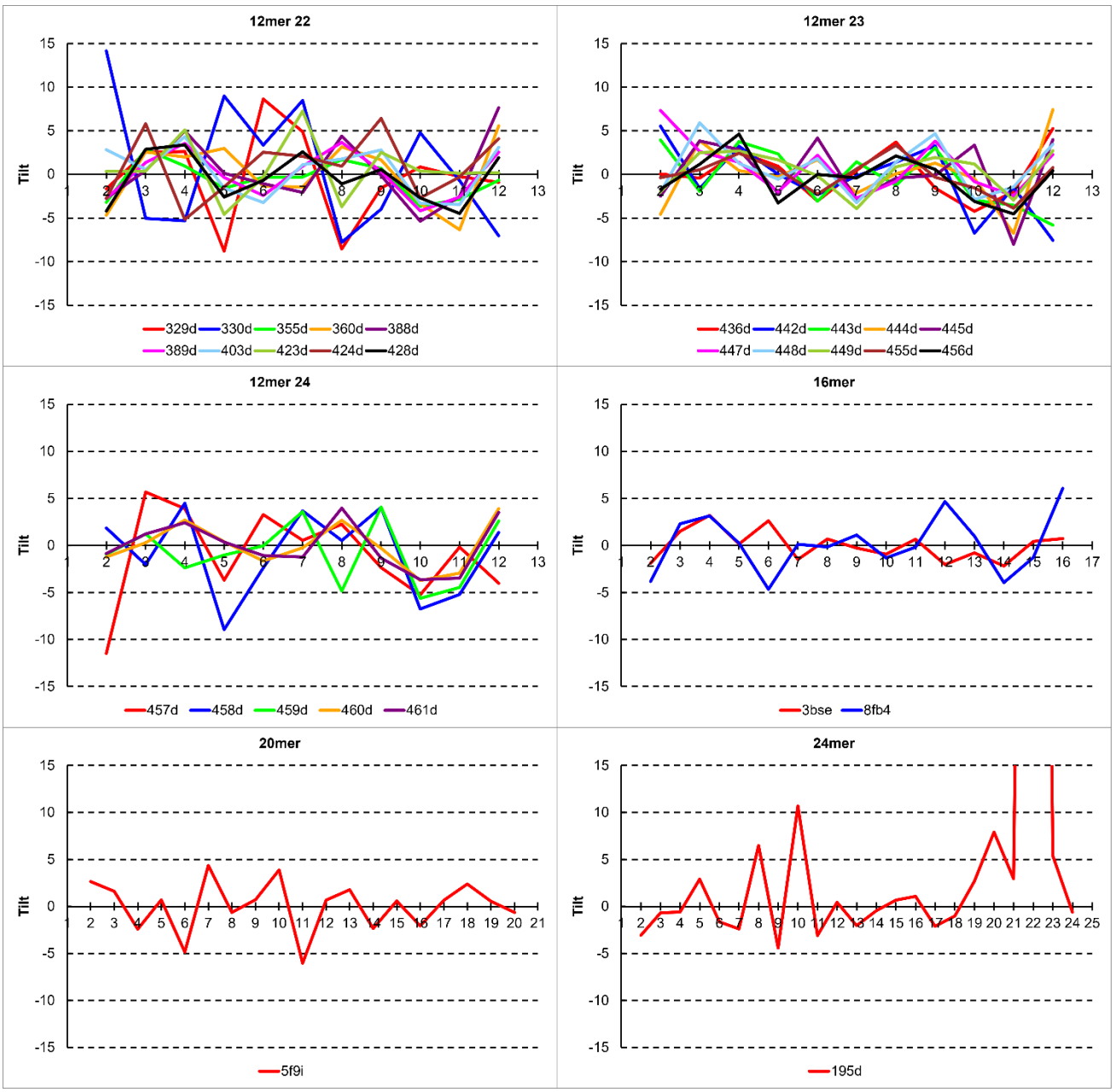
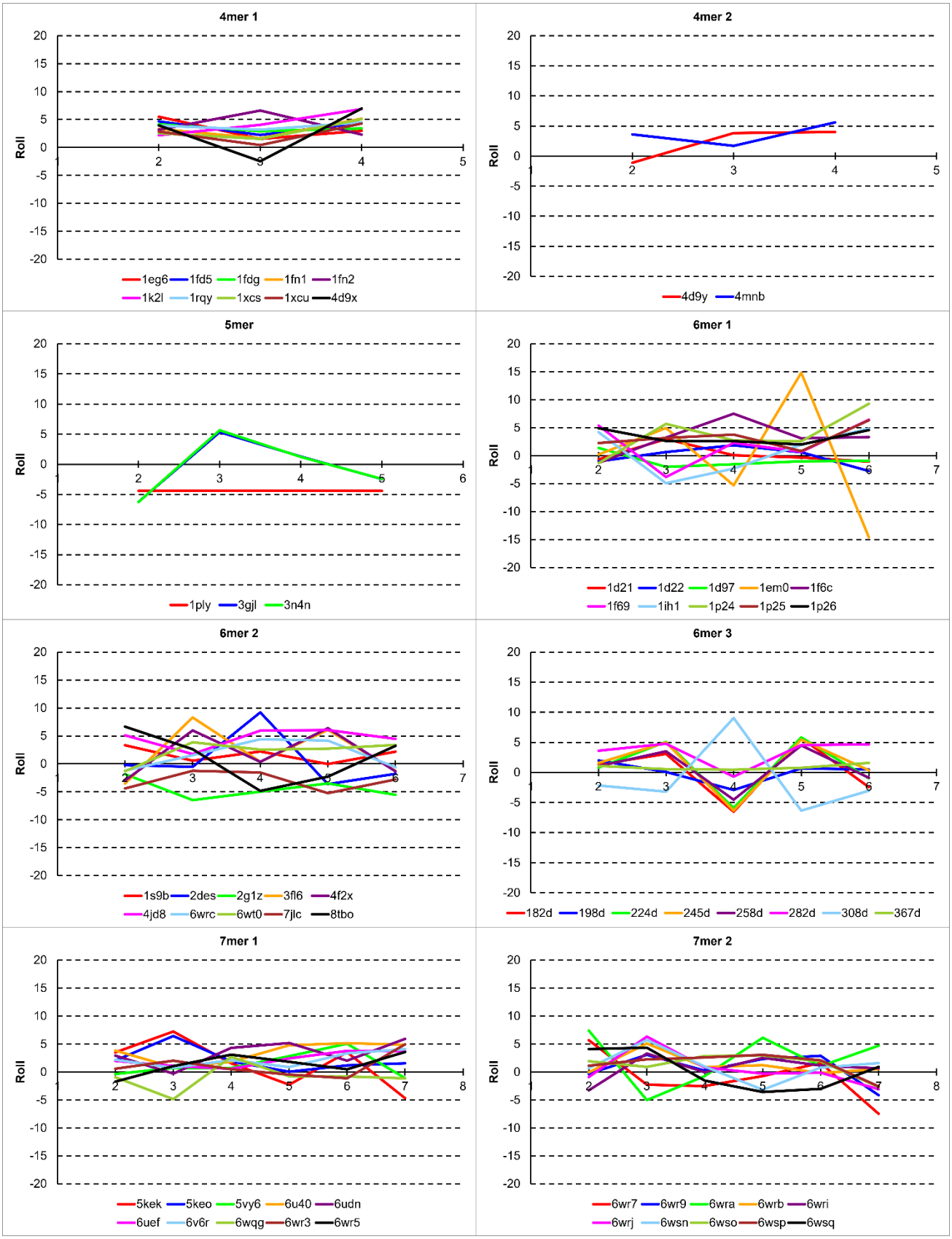
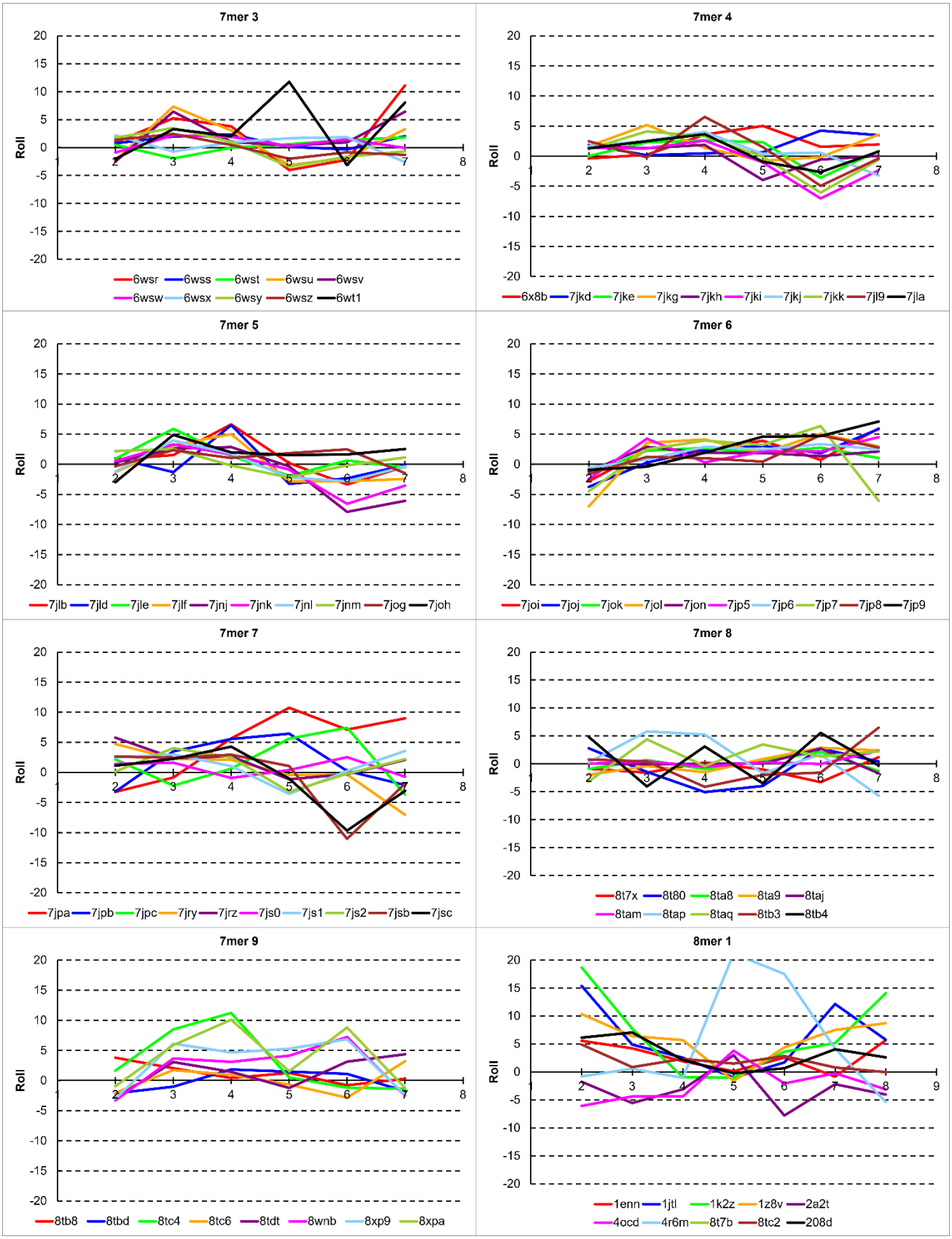
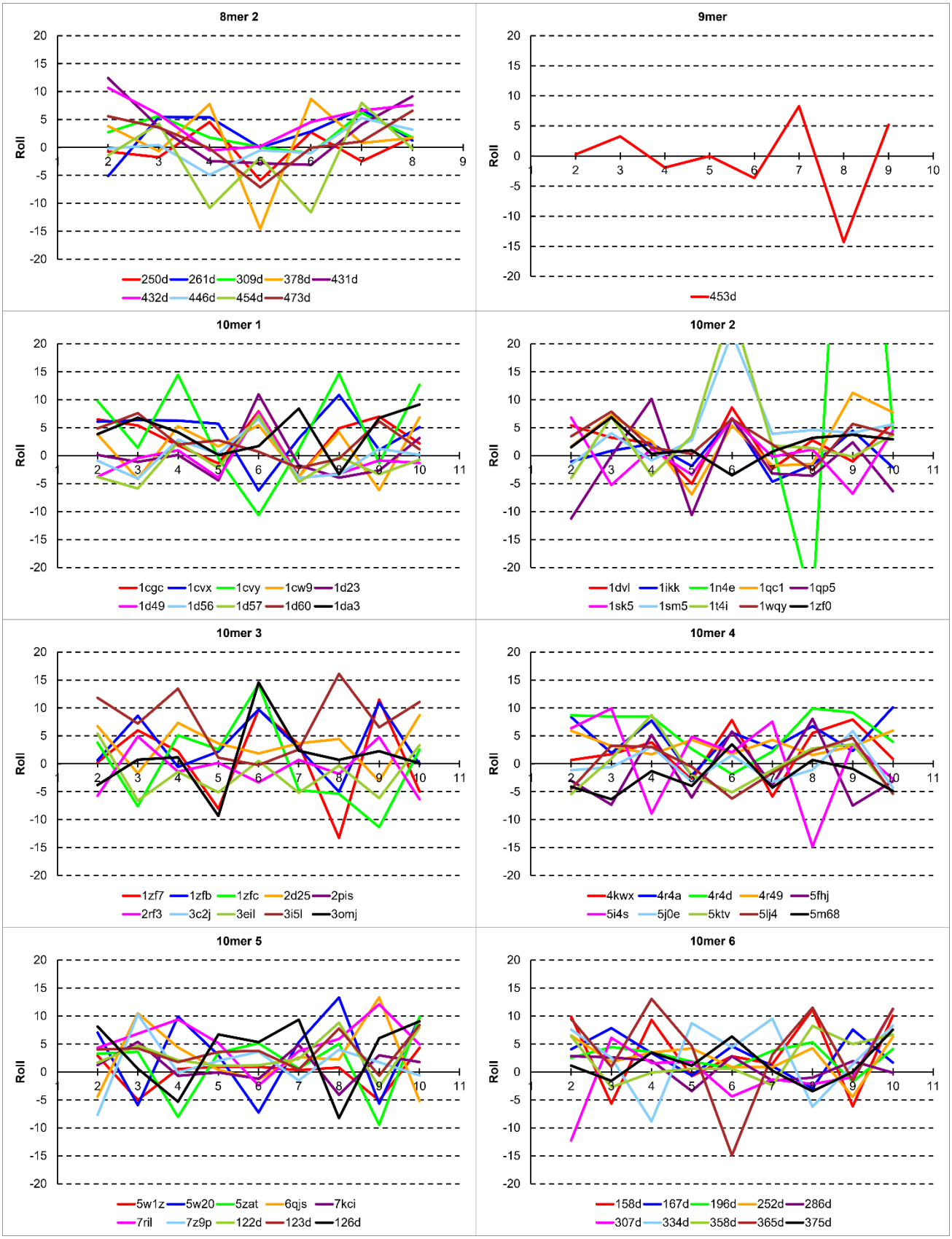
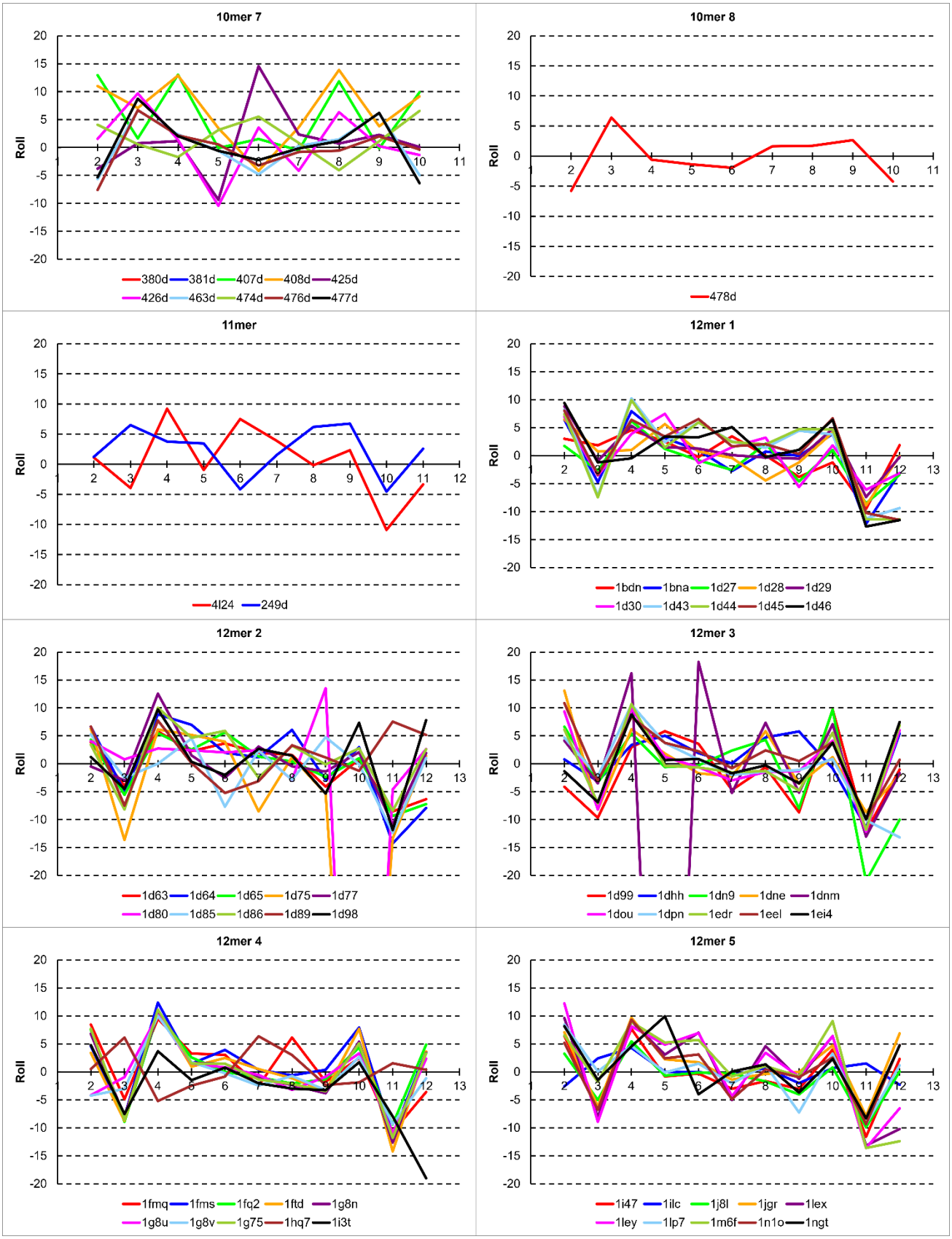

12mer 22
Tilt
329d 330d 355d 360d 388d
389d 403d 423d 424d 428d
12mer 23
436d 442d 443d 444d 445d
447d 448d 449d 455d 456d
12mer 24
457d 458d 459d 460d 461d
16mer
3bse 8fb4
20mer
5f9i
24mer
195d

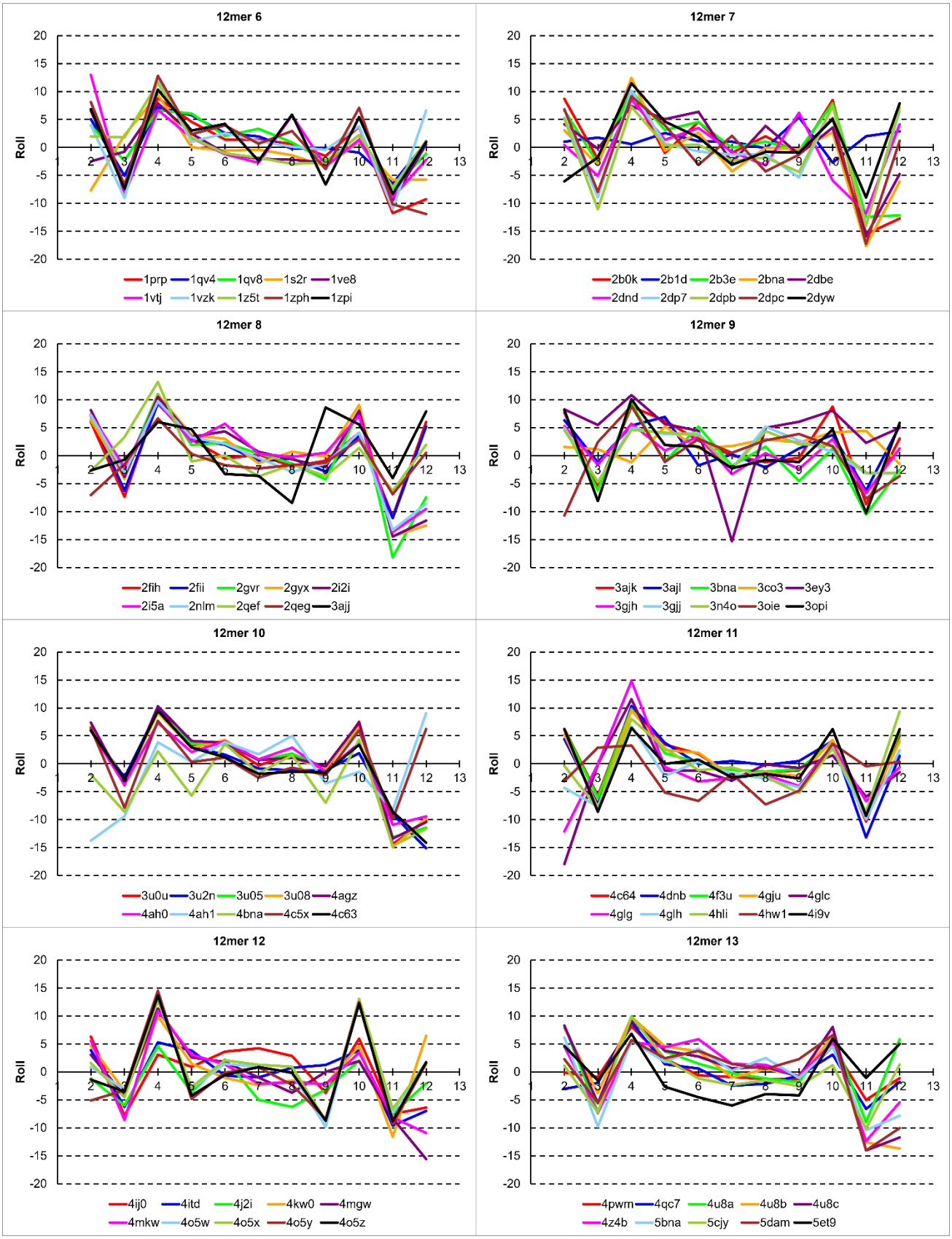
**Figure S5:** The graphs to visualize the regularity of Roll for the B-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.

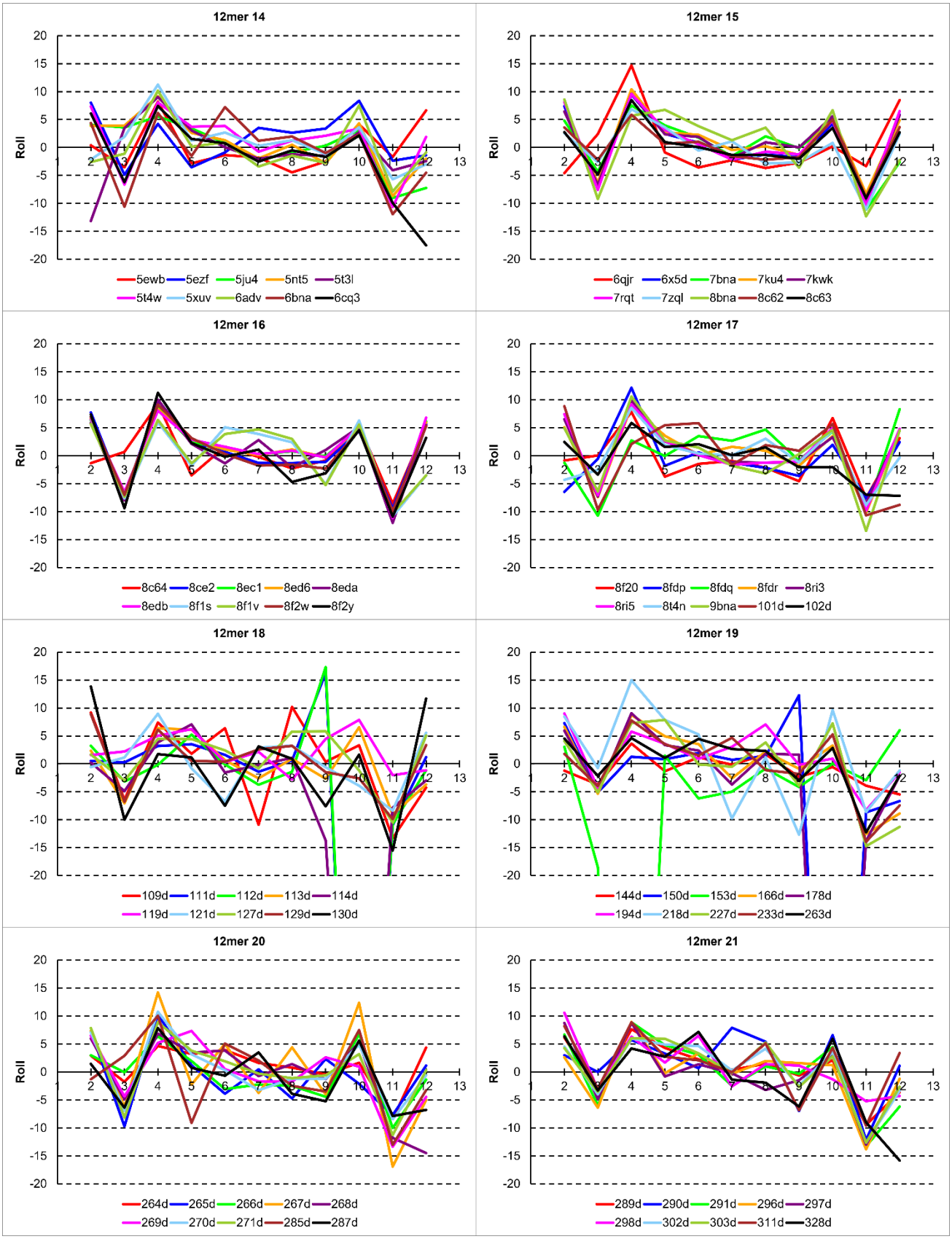



7mer 3
Roll
6wsr 6wss 6wst 6wsu 6wsv
6wsw 6wsx 6wsy 6wsz 6wt1
7mer 4
Roll
6x8b 7jkd 7jke 7jkg 7jkh 7jki 7jkj 7jkk 7jl9 7jla
7mer 5
Roll
7jlb 7jld 7jle 7jlf 7jnj 7jnk 7jnl 7jnm 7jog 7joh
7mer 6
Roll
7joi 7joj 7jok 7jol 7jon 7jp5 7jp6 7jp7 7jp8 7jp9
7mer 7
Roll
7jpa 7jpb 7jpc 7jry 7jrz 7js0 7js1 7js2 7jsb 7jsc
7mer 8
Roll
8t7x 8t80 8ta8 8ta9 8taj
8tam 8tap 8taq 8tb3 8tb4
7mer 9
Roll
8tb8 8tbd 8tc4 8tc6 8tdt 8wnb 8xp9 8xpa
8mer 1
Roll
1enn 1jtl 1k2z 1z8v 2a2t
4ocd 4r6m 8t7b 8tc2 208d


8mer 2
Roll
250d 261d 309d 378d 431d
432d 446d 454d 473d
9mer
Roll
453d
10mer 1
Roll
1cgc 1cvx 1cvy 1cw9 1d23
1d49 1d56 1d57 1d60 1da3
10mer 2
Roll
1dvl 1ikk 1n4e 1qc1 1qp5
1sk5 1sm5 1t4i 1wqy 1zf0
10mer 3
Roll
1zf7 1zfb 1zfc 2d25 2pis
2rf3 3c2j 3eil 3i5l 3omj
10mer 4
Roll
4kwx 4r4a 4r4d 4r49 5fhj
5i4s 5j0e 5ktv 5lj4 5m68
10mer 5
Roll
5w1z 5w20 5zat 6qjs 7kci
7ril 7z9p 122d 123d 126d
10mer 6
Roll
158d 167d 196d 252d 286d
307d 334d 358d 365d 375d


10mer 7
Roll
380d 381d 407d 408d 425d
426d 463d 474d 476d 477d
10mer 8
Roll
478d
11mer
Roll
4l24 249d
12mer 1
Roll
1bdn 1bna 1d27 1d28 1d29
1d30 1d43 1d44 1d45 1d46
12mer 2
Roll
1d63 1d64 1d65 1d75 1d77
1d80 1d85 1d86 1d89 1d98
12mer 3
Roll
1d99 1dhh 1dn9 1dne 1dnm
1dou 1dpn 1edr 1eel 1ei4
12mer 4
Roll
1fmq 1fms 1fq2 1ftd 1g8n
1g8u 1g8v 1g75 1hq7 1i3t
12mer 5
Roll
1i47 1ilc 1j8l 1jgr 1lex
1ley 1lp7 1m6f 1n1o 1ngt


12mer 6
Roll
1prp 1qv4 1qv8 1s2r 1ve8
1vtj 1vzk 1z5t 1zph 1zpi
12mer 7
Roll
2b0k 2b1d 2b3e 2bna 2dbe
2dnd 2dp7 2dpb 2dpc 2dyw
12mer 8
Roll
2fih 2fii 2gvr 2gyx 2i2i
2i5a 2nlm 2qef 2qeg 3ajj
12mer 9
Roll
3ajk 3ajl 3bna 3co3 3ey3
3gjh 3gjj 3n4o 3oie 3opi
12mer 10
Roll
3u0u 3u2n 3u05 3u08 4agz
4ah0 4ah1 4bna 4c5x 4c63
12mer 11
Roll
4c64 4dnb 4f3u 4gju 4glc
4glg 4glh 4hli 4hw1 4i9v
12mer 12
Roll
4ij0 4itd 4j2i 4kw0 4mgw
4mkw 4o5w 4o5x 4o5y 4o5z
12mer 13
Roll
4pwm 4qc7 4u8a 4u8b 4u8c
4z4b 5bna 5cjy 5dam 5et9


12mer 14
Roll
5ewb 5ezf 5ju4 5nt5 5t3l
5t4w 5xuv 6adv 6bna 6cq3
12mer 15
Roll
6qjr 6x5d 7bna 7ku4 7kwk
7rqt 7zql 8bna 8c62 8c63
12mer 16
Roll
8c64 8ce2 8ec1 8ed6 8eda
8edb 8f1s 8f1v 8f2w 8f2y
12mer 17
Roll
8f20 8fdp 8fdq 8fdr 8ri3
8ri5 8t4n 9bna 101d 102d
12mer 18
Roll
109d 111d 112d 113d 114d
119d 121d 127d 129d 130d
12mer 19
Roll
144d 150d 153d 166d 178d
194d 218d 227d 233d 263d
12mer 20
Roll
264d 265d 266d 267d 268d
269d 270d 271d 285d 287d
12mer 21
Roll
289d 290d 291d 296d 297d
298d 302d 303d 311d 328d

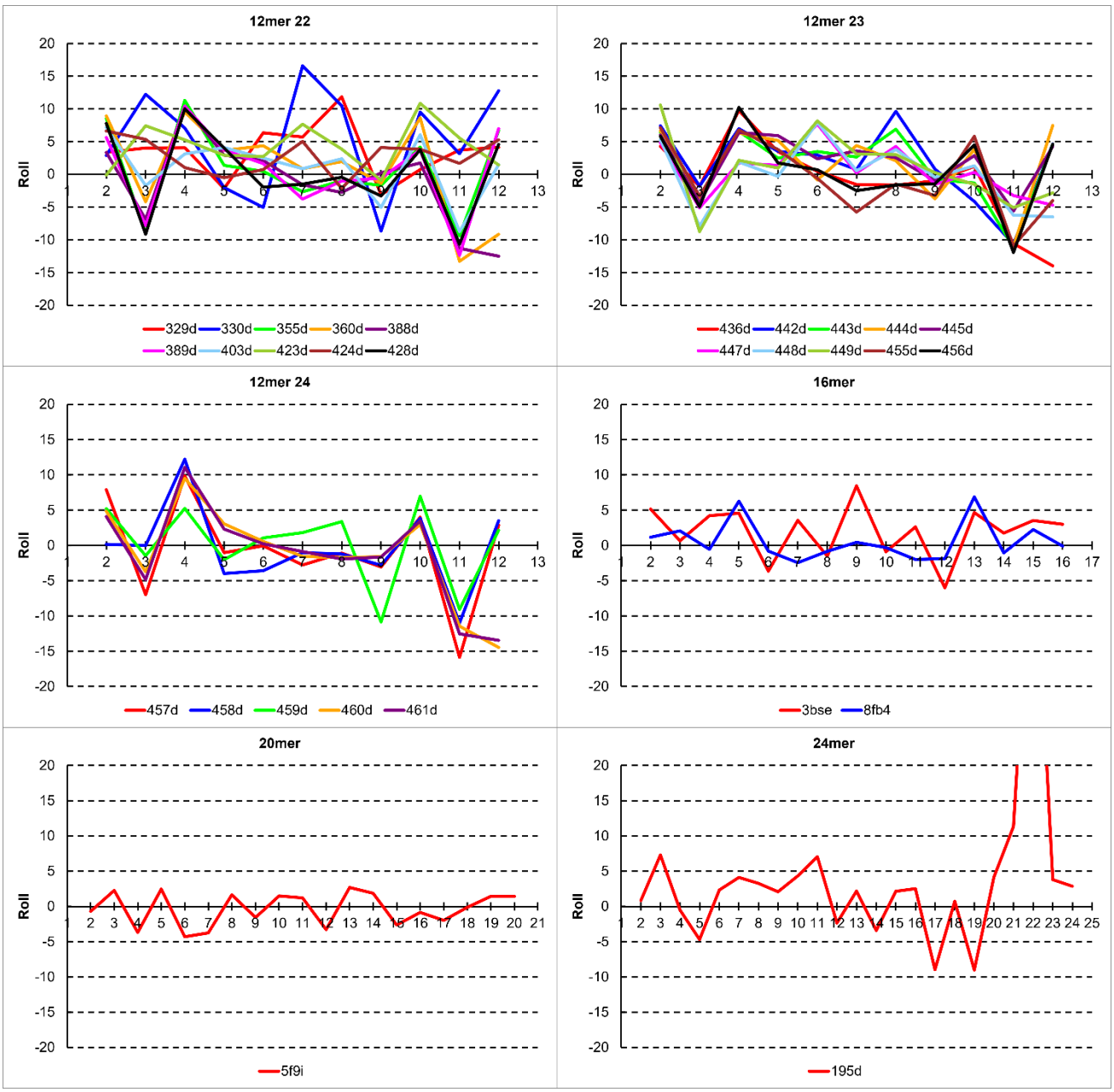
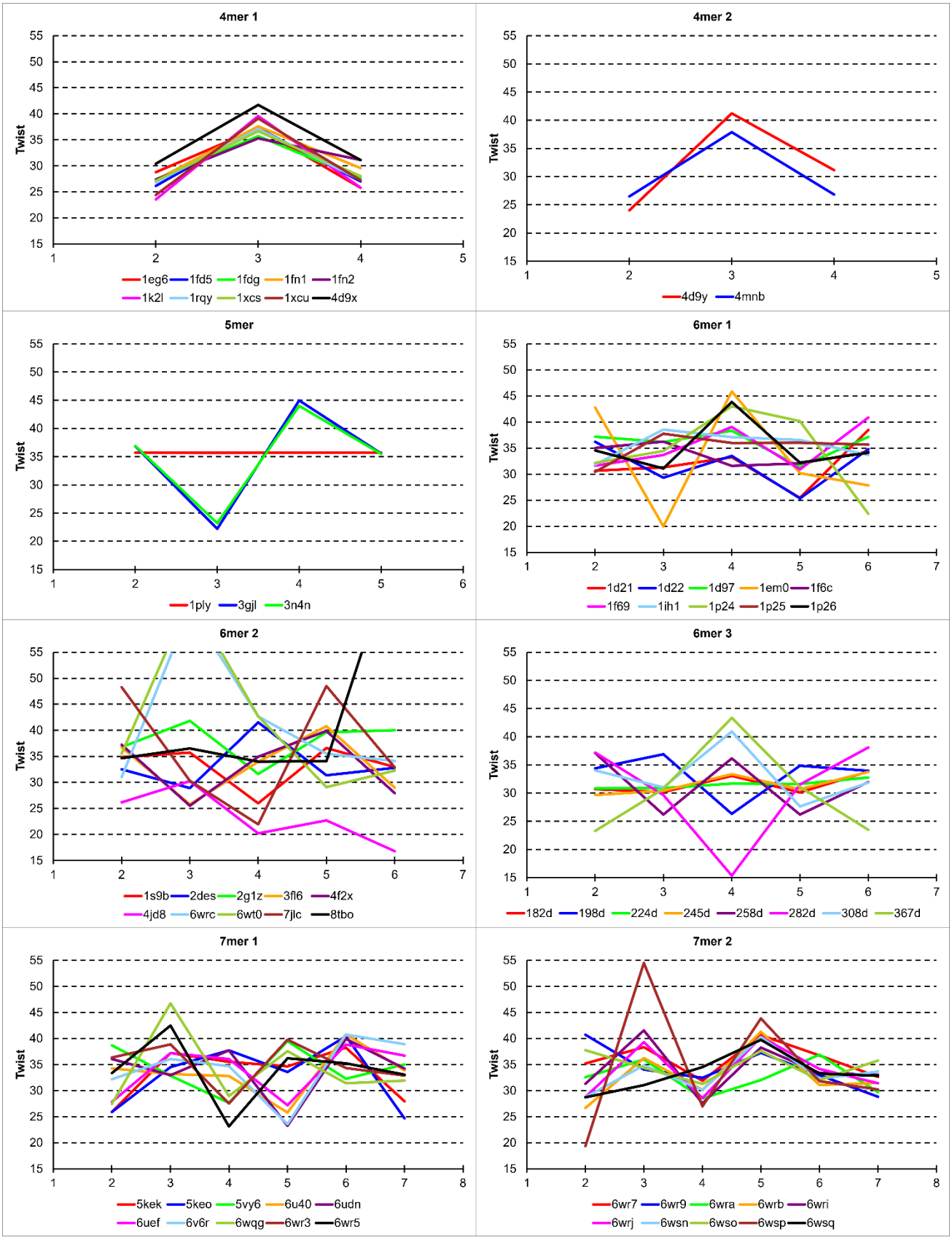
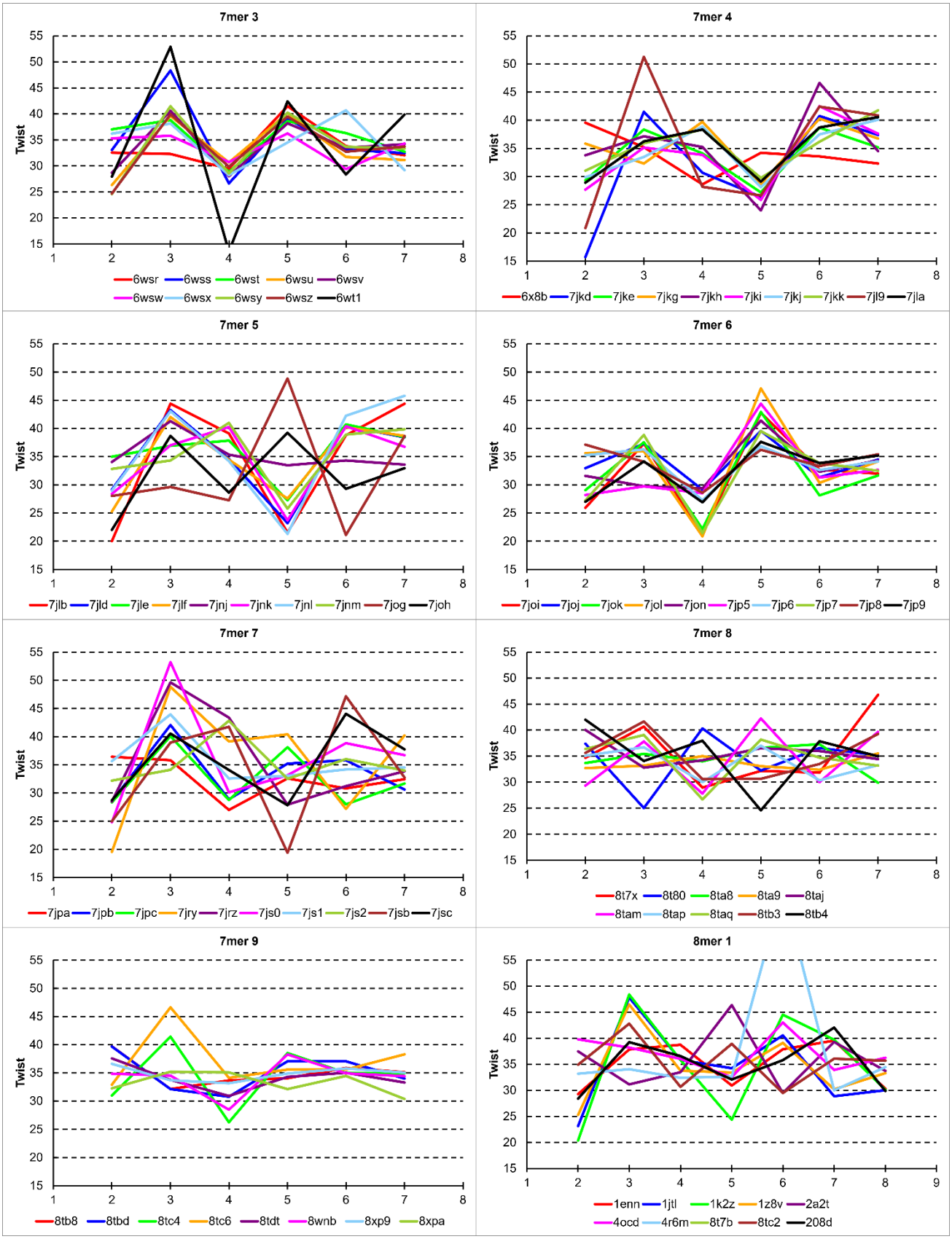
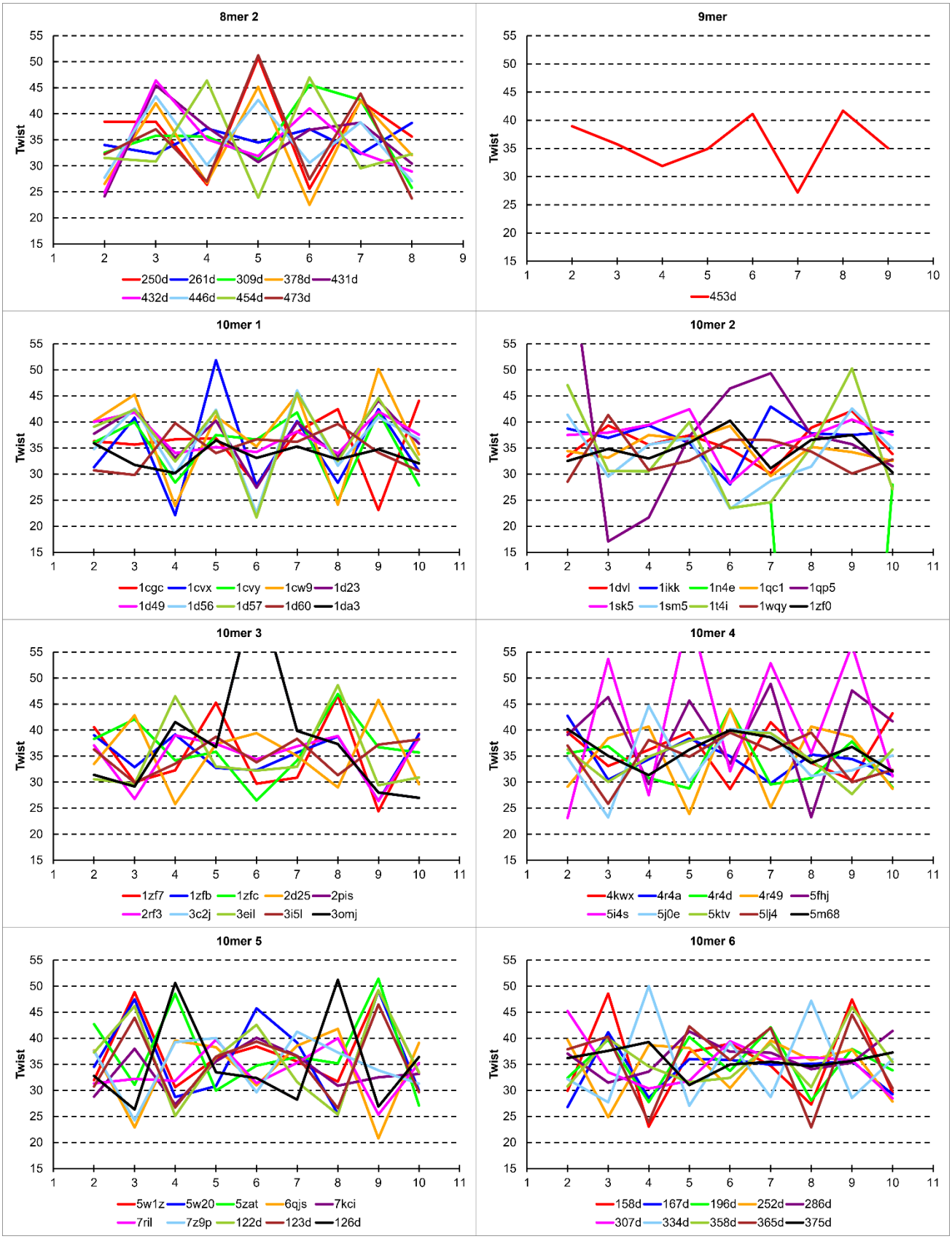
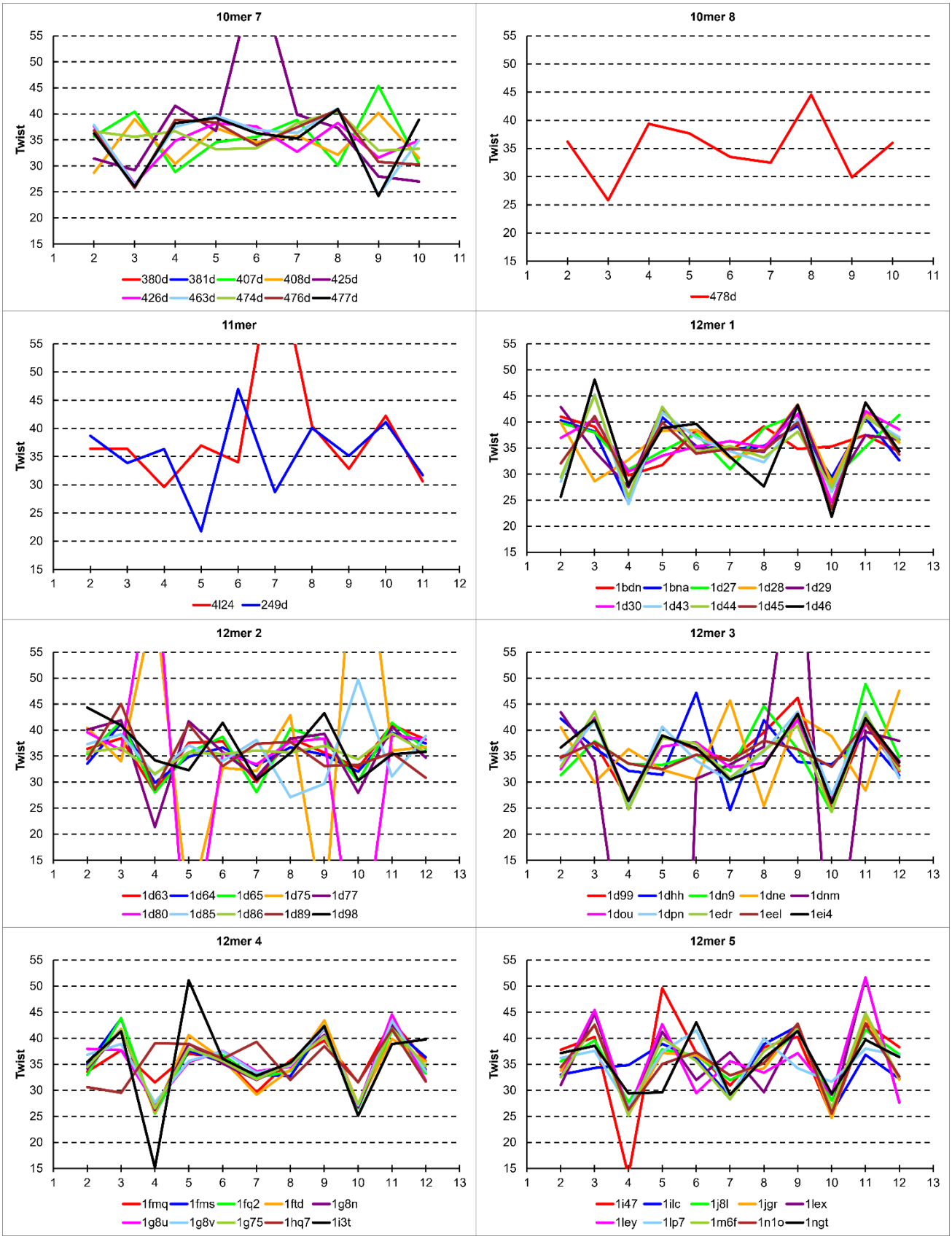

12mer 22
Roll
329d 330d 355d 360d 388d
389d 403d 423d 424d 428d
12mer 23
Roll
436d 442d 443d 444d 445d
447d 448d 449d 455d 456d
12mer 24
Roll
457d 458d 459d 460d 461d
16mer
Roll
3bse 8fb4
20mer
Roll
5f9i
24mer
Roll
195d

**Figure S6:** The graphs to visualize the regularity of Twist for the B-form duplexes. Since the scale intervals on the vertical axis are the same for all graphs. The horizontal axis corresponds to base-pair steps, where the value at position $i$ represents the parameter between base pairs $i-1$ and $i$.




7mer 3
Twist
6wsr 6wss 6wst 6wsu 6wsv
6wsw 6wsx 6wsy 6wsz 6wt1
7mer 4
Twist
6x8b 7jkd 7jke 7jkg 7jkh 7jki 7jkj 7jkk 7jl9 7jla
7mer 5
Twist
7jlb 7jld 7jle 7jlf 7jnj 7jnk 7jnl 7jnm 7jog 7joh
7mer 6
Twist
7joi 7joj 7jok 7jol 7jon 7jp5 7jp6 7jp7 7jp8 7jp9
7mer 7
Twist
7jpa 7jpb 7jpc 7jry 7jrz 7js0 7js1 7js2 7jsb 7jsc
7mer 8
Twist
8t7x 8t80 8ta8 8ta9 8taj
8tam 8tap 8taq 8tb3 8tb4
7mer 9
Twist
8tb8 8tbd 8tc4 8tc6 8tdt 8wnb 8xp9 8xpa
8mer 1
Twist
1enn 1jtl 1k2z 1z8v 2a2t
4ocd 4r6m 8t7b 8tc2 208d


8mer 2
Twist
250d 261d 309d 378d 431d
432d 446d 454d 473d
9mer
Twist
453d
10mer 1
Twist
1cgc 1cvx 1cvy 1cw9 1d23
1d49 1d56 1d57 1d60 1da3
10mer 2
Twist
1dvl 1ikk 1n4e 1qc1 1qp5
1sk5 1sm5 1t4i 1wqy 1zf0
10mer 3
Twist
1zf7 1zfb 1zfc 2d25 2pis
2rf3 3c2j 3eil 3i5l 3omj
10mer 4
Twist
4kwx 4r4a 4r4d 4r49 5fhj
5i4s 5j0e 5ktv 5lj4 5m68
10mer 5
Twist
5w1z 5w20 5zat 6qjs 7kci
7ril 7z9p 122d 123d 126d
10mer 6
Twist
158d 167d 196d 252d 286d
307d 334d 358d 365d 375d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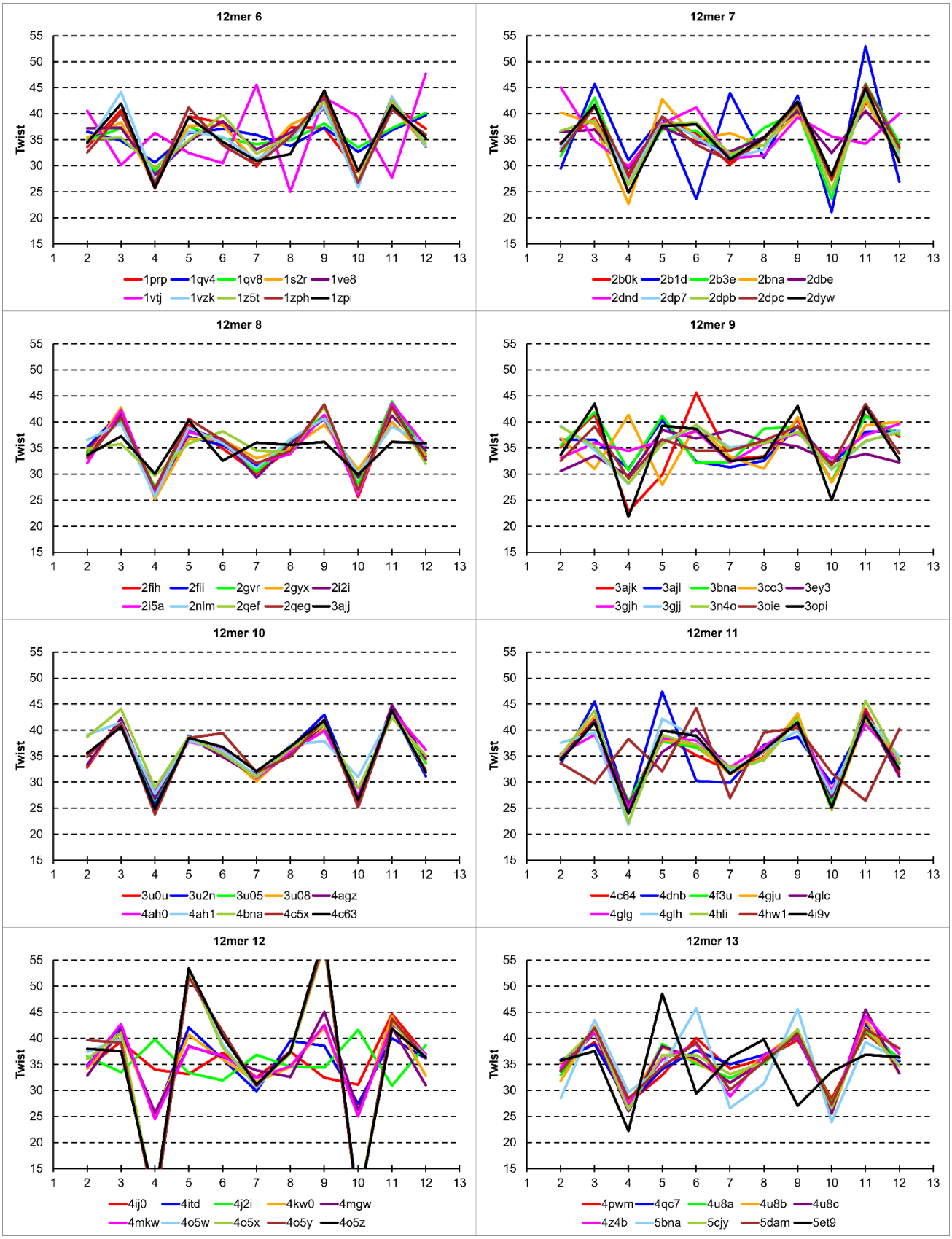
426d 463d 474d 476d 477d
10mer 8
Twist
478d
11mer
Twist
4l24 249d
12mer 1
Twist
1bdn 1bna 1d27 1d28 1d29
1d30 1d43 1d44 1d45 1d46
12mer 2
Twist
1d63 1d64 1d65 1d75 1d77
1d80 1d85 1d86 1d89 1d98
12mer 3
Twist
1d99 1dhh 1dn9 1dne 1dnm
1dou 1dpn 1edr 1eel 1ei4
12mer 4
Twist
1fmq 1fms 1fq2 1ftd 1g8n
1g8u 1g8v 1g75 1hq7 1i3t
12mer 5
Twist
1i47 1ilc 1j8l 1jgr 1lex
1ley 1lp7 1m6f 1n1o 1ngt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Twist
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Twist
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2dnd 2dp7 2dpb 2dpc 2dyw
12mer 8
Twist
2fih 2fii 2gvr 2gyx 2i2i
2i5a 2nlm 2qef 2qeg 3ajj
12mer 9
Twist
3ajk 3ajl 3bna 3co3 3ey3
3gjh 3gjj 3n4o 3oie 3opi
12mer 10
Twist
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4ah0 4ah1 4bna 4c5x 4c63
12mer 11
Twist
4c64 4dnb 4f3u 4gju 4glc
4glg 4glh 4hli 4hw1 4i9v
12mer 12
Twist
4ij0 4itd 4j2i 4kw0 4mgw
4mkw 4o5w 4o5x 4o5y 4o5z
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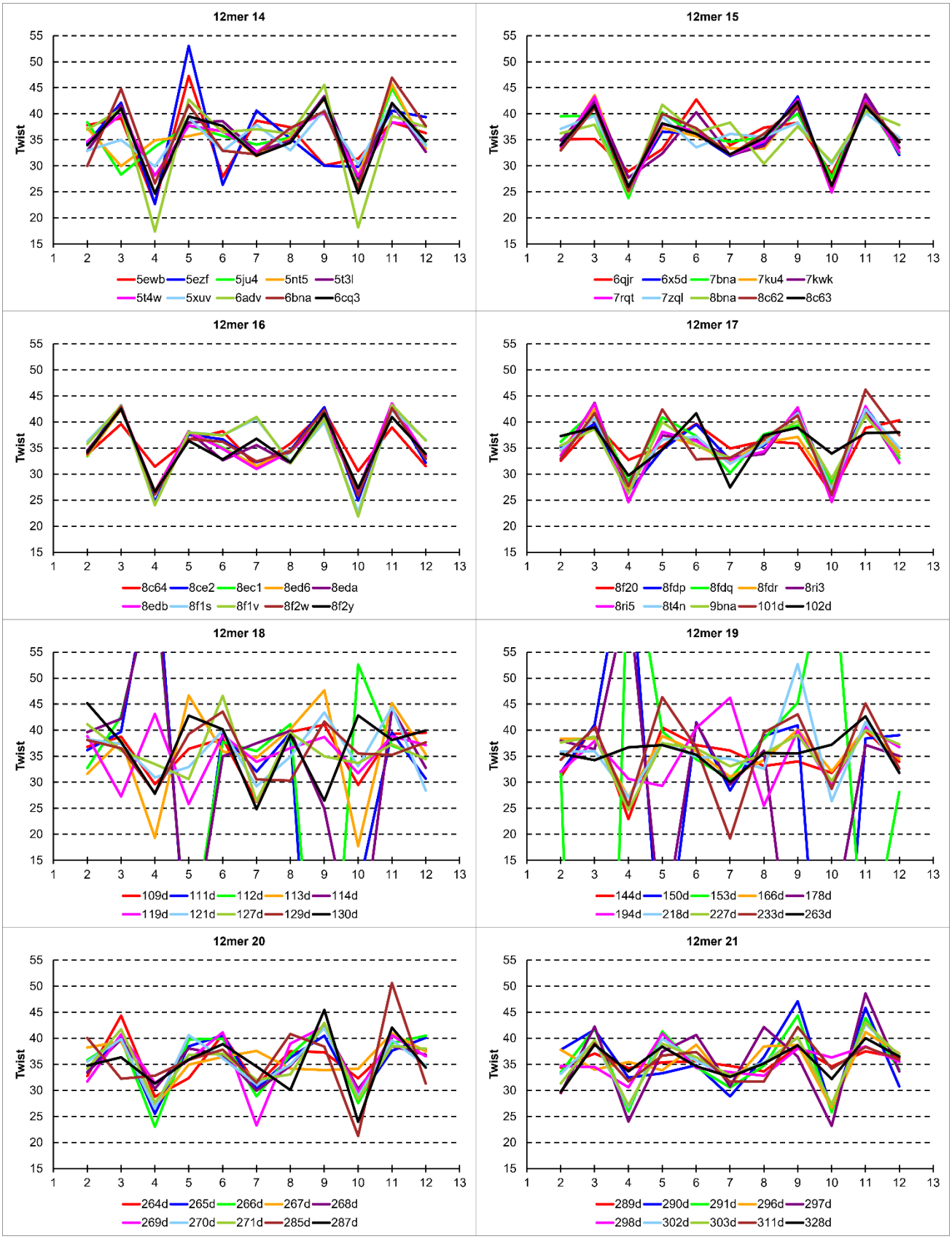
4z4b 5bna 5cjy 5dam 5et9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Twist
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5t4w 5xuv 6adv 6bna 6cq3
12mer 15
Twist
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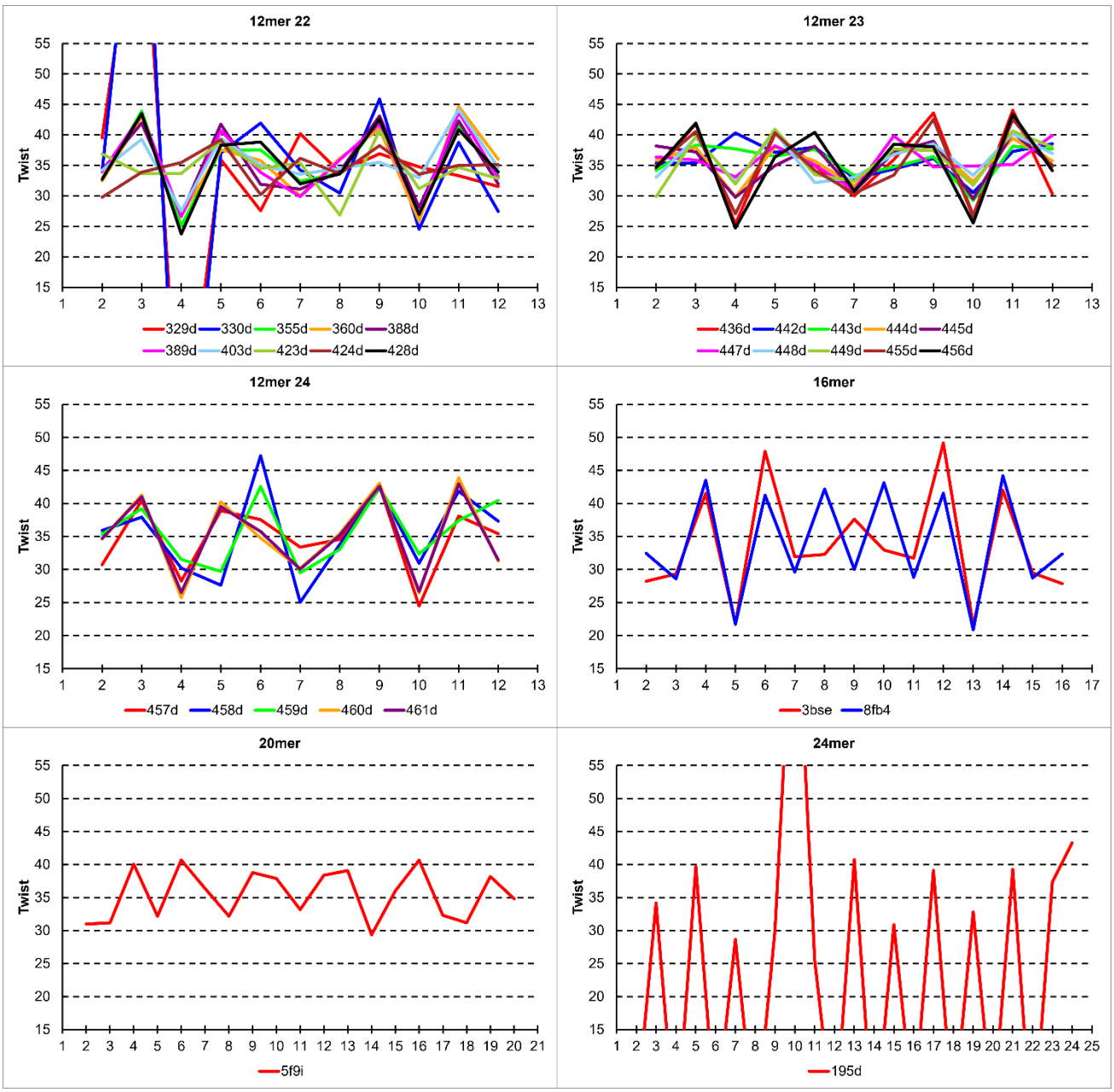
7rqt 7zql 8bna 8c62 8c63
12mer 16
Twist
8c64 8ce2 8ec1 8ed6 8eda
8edb 8f1s 8f1v 8f2w 8f2y
12mer 17
Twist
8f20 8fdp 8fdq 8fdr 8ri3
8ri5 8t4n 9bna 101d 102d
12mer 18
Twist
109d 111d 112d 113d 114d
119d 121d 127d 129d 130d
12mer 19
Twist
144d 150d 153d 166d 178d
194d 218d 227d 233d 263d
12mer 20
Twist
264d 265d 266d 267d 268d
269d 270d 271d 285d 287d
12mer 21
Twist
289d 290d 291d 296d 297d
298d 302d 303d 311d 328d


12mer 22
Twist
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389d 403d 423d 424d 428d
12mer 23
436d 442d 443d 444d 445d
447d 448d 449d 455d 456d
12mer 24
457d 458d 459d 460d 461d
16mer
3bse 8fb4
20mer
5f9i
24mer
195d

**Figure S7:** The electron density map obtained after one round of refinement following MR using the ideal A-form duplex model for the test case 257D.

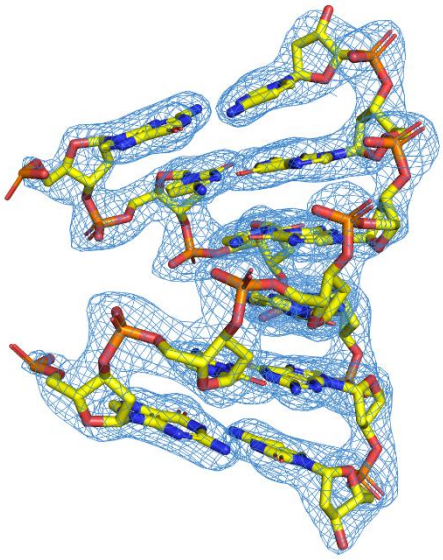

**Figure S8:** The electron density maps and models at each step after MR using the ideal A-form duplex model for the test case 1T0E. **(A)** MR solution obtained using the ideal A-form duplex model generated by the software *Coot*. The overall position of the model was approximately correct, but the model did not fit the electron density closely. **(B)** Model after one round of refinement. Since the model showed reasonable agreement with the electron density map, the structure was judged to be solvable through repeated cycles of refinement and model correction. **(C)** Model after retaining only the regions that fit the electron density and performing further refinement. The backbone fitted the electron density well, but the bases did not fit appropriately, suggesting that the model was placed in the opposite orientation. **(D)** Model after reversing the orientation by exchanging the two strands and building the loop region. Refinement could be completed by additional minor corrections, such as the placement of water molecules.

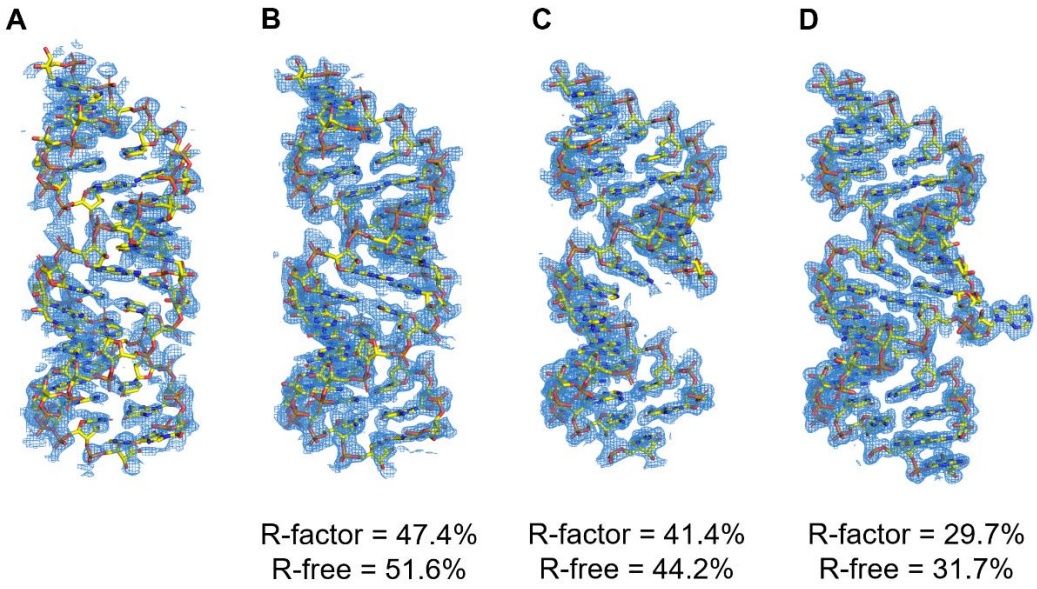

**Figure S9:** The electron density map obtained after MR with 4MRNA for the test case 3TD0. The electron density of the loop region is clearly visible **(A)**, which enabled easy placement of the nucleotide **(B)**.

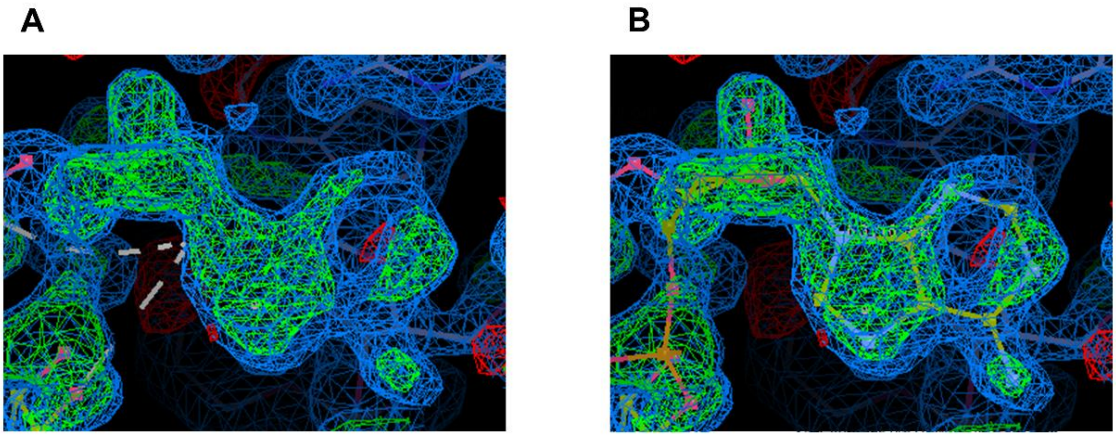

**Figure S10:** The electron density map obtained after loop placement and refinement for the test case 3TD1. The central green mesh clearly shows the electron density of the ligand.

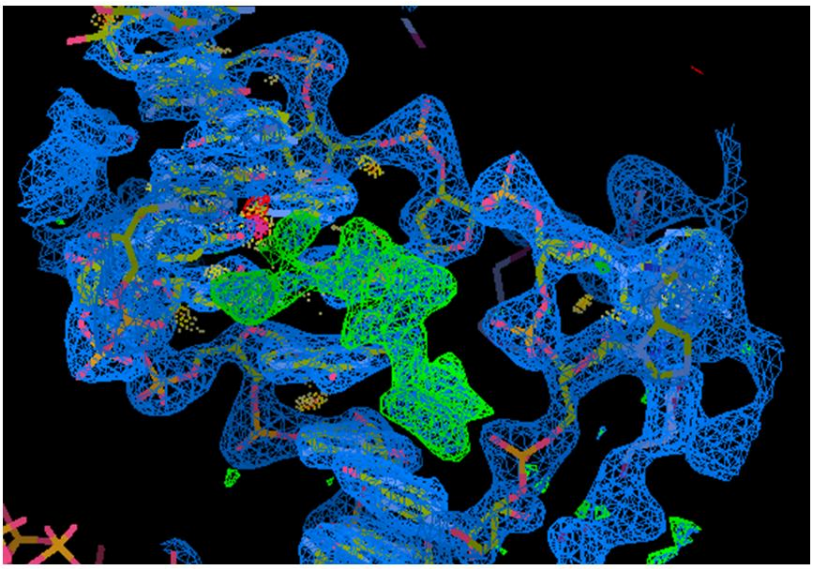

**Figure S11:** Results of several conventional MR approaches applied to the test case 3TD0. **(A)** The electron density map obtained after one round of refinement following MR using 1MWL. The model did not fit the electron density in most regions, and some parts of the model clashed with symmetry-related molecules. **(B)** The electron density map obtained after one round of refinement following MR using 3TD0. The model did not fit the electron density in most regions. **(C)** The electron density map obtained after one round of refinement following MR using ideal A-form duplex models generated by the software *Coot*. Three stem fragments were combined according to the same sequence partitioning shown in Figure 8. The models did not fit the electron density in most regions, and the stem models overlapped sterically with each other.

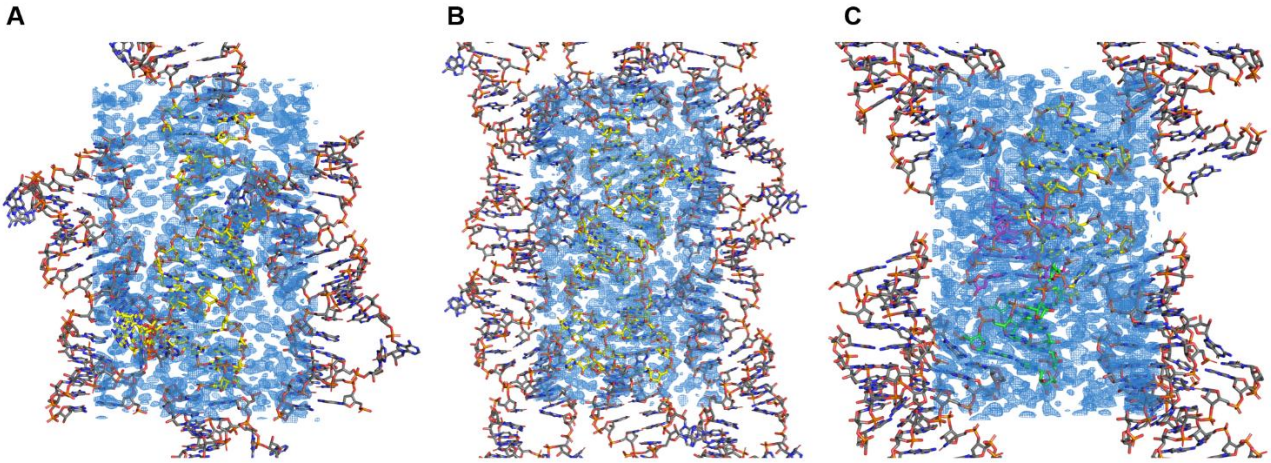

**Figure S12:** Secondary and tertiary structures of tRNA (PDB ID 6TNA). 6TNA is the first solved tRNA structure (modified version), and other tRNAs generally have similar structures. **(A)** Secondary structure prediction using *MXFold2*. **(B)** Three-dimensional structure. The stem of the acceptor arm and the stem of the T arm are connected together to form one duplex.

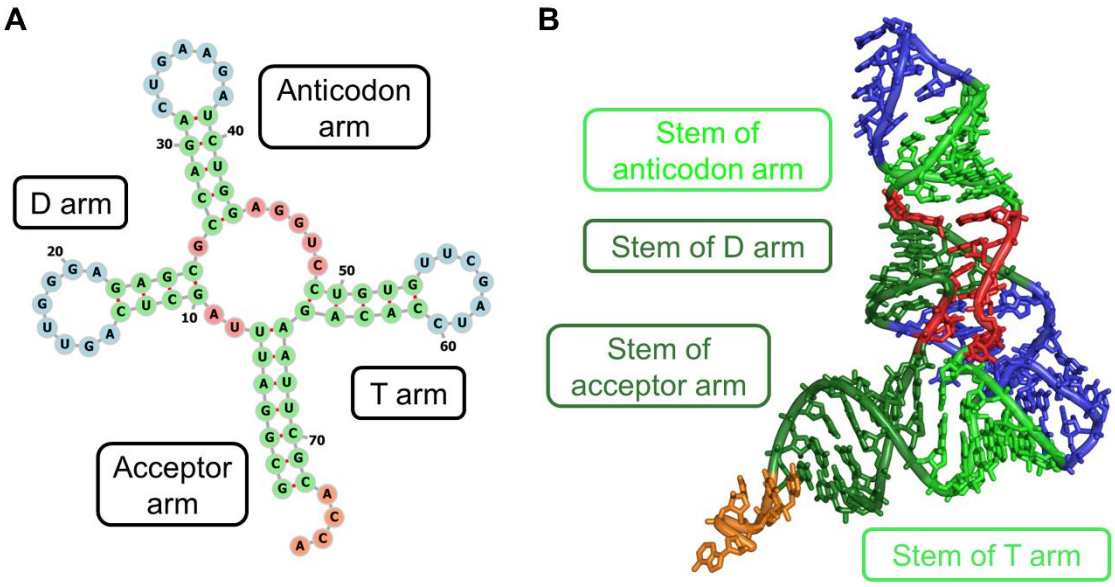

**Figure S13:** Loop modelling in the test case 2TRA. Panels **(A)**-**(E)** show the stepwise process of loop placement and refinement. Nucleotides were positioned where electron density was clearly visible, and successive rounds of refinement progressively improved the density of neighboring regions. Through this iterative process, nucleotides in the loop could be placed step by step, leading to structure determination.

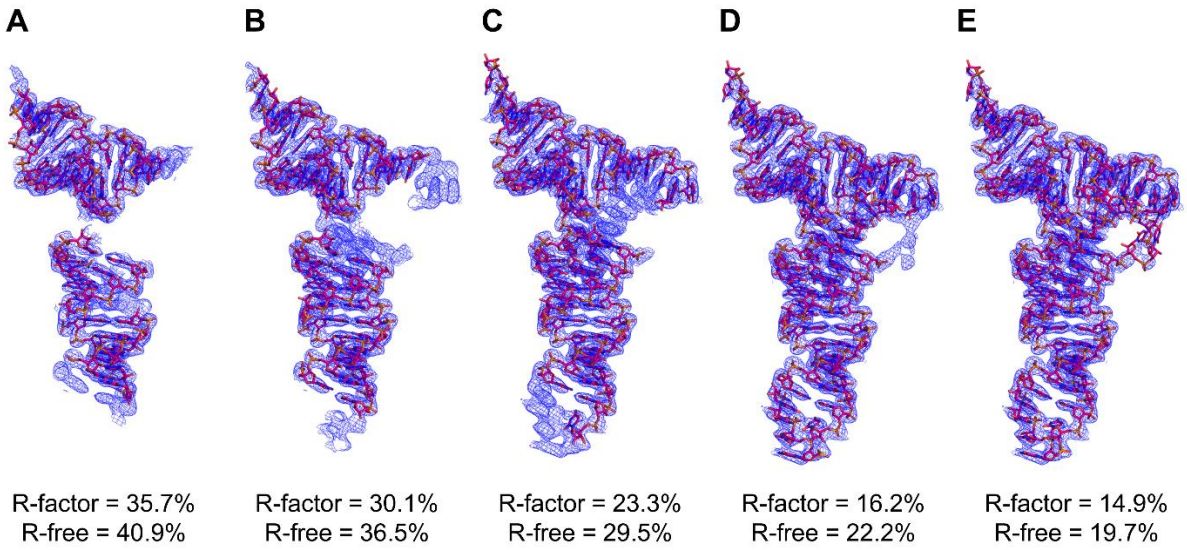

**Figure S14:** Results of several conventional MR approaches applied to the test case 2TRA. **(A)** The electron density map obtained after one round of refinement following MR using 6TNA. The model did not fit the electron density in most regions, and some parts of the model clashed with symmetry-related molecules. **(B)** Secondary structure and model partitioning. **(C)** The electron density map obtained after one round of refinement following MR using ideal A-form duplex models generated by the software *Coot*. Three stem fragments were combined according to the same sequence partitioning shown in Figure 9. The models did not fit the electron density in most regions, and the stem models overlapped sterically with each other. **(D)** The electron density map obtained after one round of refinement following MR using an ideal AT-duplex model, an ideal D-duplex model, and an anticodon arm model. The anticodon arm model was prepared by extracting the anticodon loop region from 6TNA and extending both strands from this loop region using the software *Coot*. The models did not fit the electron density in most regions, and the stem models overlapped sterically with each other, and some parts of the model clashed with symmetry-related molecules.

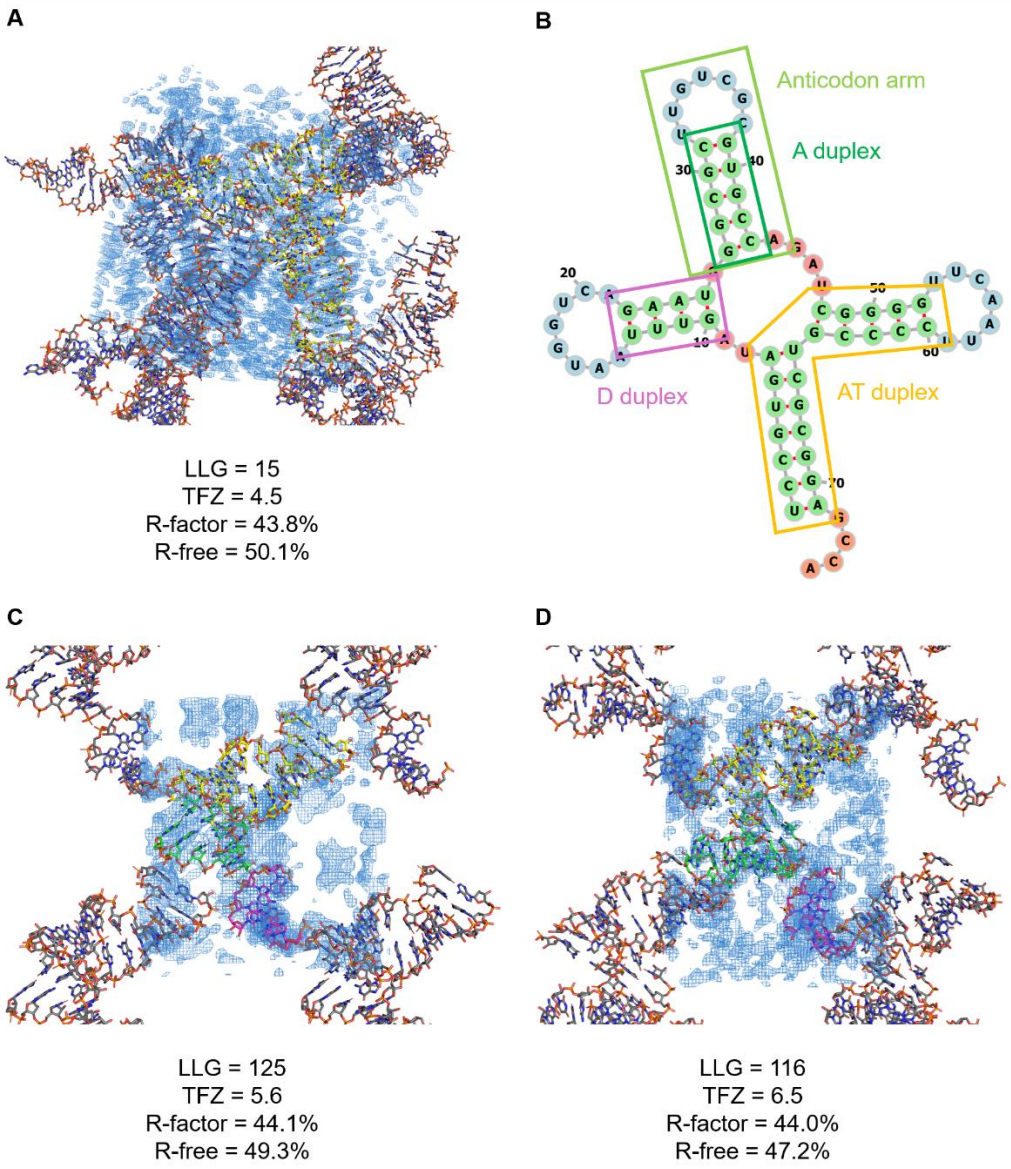

**Figure S15:** Result of MR using the ideal A-form duplex model for the unreported structure. **(A, B)** The electron density map obtained after one round of refinement following MR using an ideal A-form duplex model generated by the software *Coot*, viewed from two directions. The models did not fit the electron density in most regions, and the stem models overlapped sterically with each other, and some parts of the model clashed with symmetry-related molecules. **(C, D)** Superimposition diagrams of the MR result using an ideal A-form duplex model and the final refined structure viewed from two directions. The final refined structure is shown in gray, whereas the three molecules in the MR result are shown in different colors. The overall RMSD was 5.659 Å. The models were placed away from their correct positions. **(E, F)** Superimposition diagrams of the MR result using an ideal A-form duplex model, the best MR model, and the final refined structure, viewed from two directions. The final refined structure is shown in gray, and the four molecules in the best MR result are shown in different colors (magenta, cyan, green, and orange), and the three molecules in the MR result are shown in different colors (red, blue, yellow).

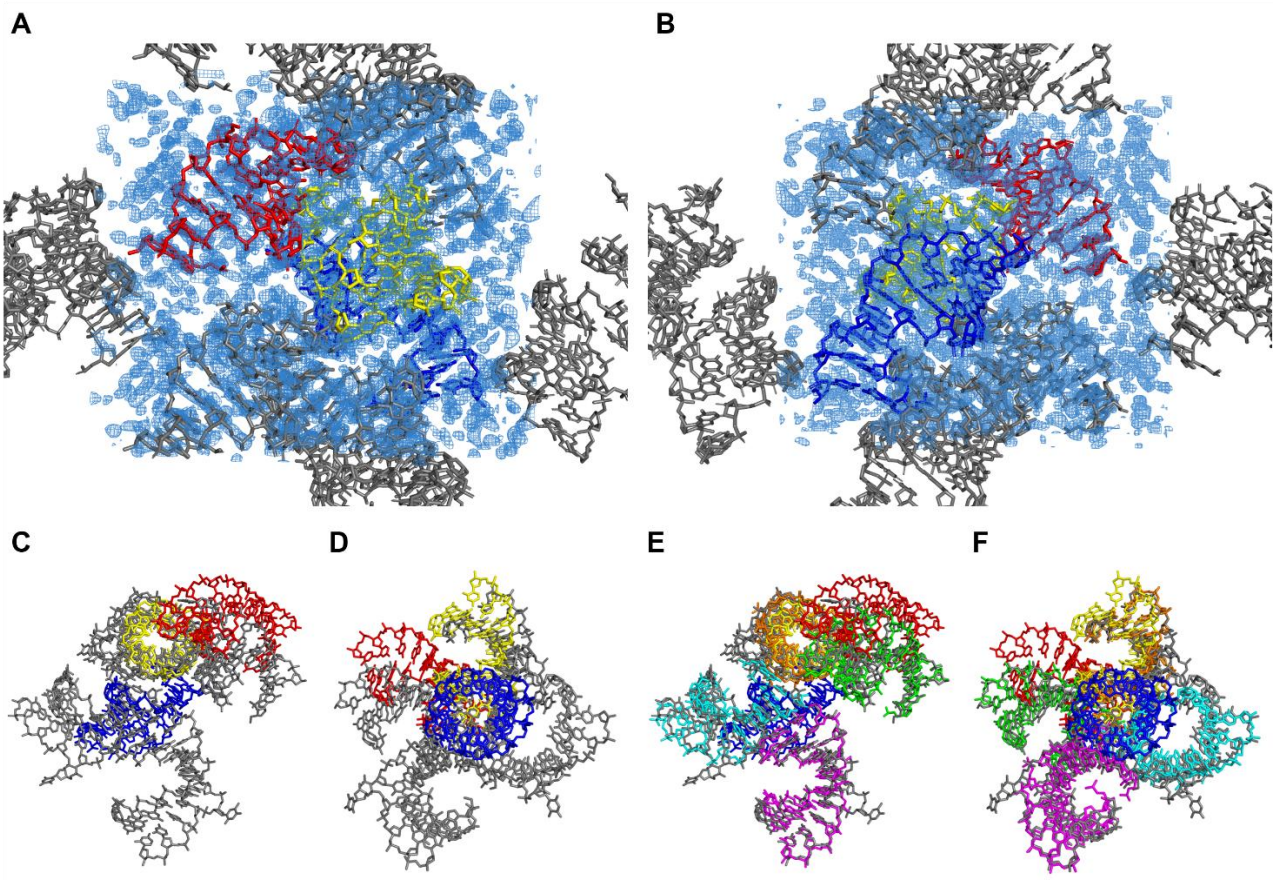

**Table S1:** A list of structures having symmetrical parameter patterns. Those whose patterns of increase or decrease from the center to the edge matched by 80% or more in both directions are listed. Non-self-complementary sequences are underlined. The total number of structures inside parentheses.

| | A-form | B-form |
|---|---|---|
| Tilt | 115d, 1i0p, 1qbp, 1vt7, 1vta, 1xjx, 1z7f, 1zf8, 1zfa, 254d, 257d, 280d, 2a0p, 2b1b, 2dqp, 2dqq, 2g3s, 2pkv, 2pl4, 2plb, 2r1s, 2r21, 319d, 343d, 3ana, 3ok2, 3r1c, 3r1d, 464d, 468d, 4e59, 4e6b, 4mce, 6c8e, 6cxz, 6gn2, 6gn3, 7ecl, 7ecn, 7ooo, 7oos, 8sx6 (42) | 111d, 112d, 126d, 129d, 144d, 150d, 1d29, 1d46, 1dne, 1eg6, 1f69, 1fd5, 1fdg, 1fn1, 1fn2, 1hq7, 1k2l, 1lp7, 1p24, 1rqy, 1t4i, 1vtj, 1xcs, 1xcu, 269d, 2bna, 2dnd, 308d, 3gjj, 432d, 446d, 457d, 4bna, 4d9x, 4d9y, 4hli, 4mnb, 5bna, 5nt5, 6qjr, 6wqg, 6wr3, 6wrc, 6wsn, 6wss, 6wst, 7jke, 7jkg, 7jkk, 7jnj, 7jol, 7jon, 7jp8, 7jry, 7js0, 7js1, 7js2, 7jsb, 8t7b, 8t80, 8ta8, 8taq, 8tb8, 8tc2 (64) |
| Roll | 115d, 157d, 1d91, 1dc0, 1dpl, 1f6e, 1i0f, 1i0g, 1i0j, 1i0k, 1i0m, 1i0n, 1i0p, 1i0q, 1i5w, 1i7j, 1ih3, 1ih4, 1ih6, 1kgk, 1ma8, 1mlx, 1nzg, 1qbp, 1r3g, 1saq, 1sdr, 1vj4, 1vta, 1wv5, 1xuw, 1xux, 1y7f, 1y84, 1y86, 1y8l, 1y8v, 1y9f, 1y9s, 1yb9, 1ybc, 1yzd, 1zf1, 1zfa, 221d, 240d, 254d, 257d, 275d, 2ao5, 2axb, 2g91, 2g92, 2gun, 2pkv, 2pl4, 2pl8, 2plb, 2plo, 2q1o, 2r1s, 2r20, 2val, 2w89, 310d, 319d, 343d, 353d, 377d, 383d, 3cjz, 3ey2, 3jxq, 3jxr, 3nj7, 3oz3, 3oz4, 3oz5, 3p4d, 3q61, 3s49, 3sd8, 3ukc, 3v06, 3v07, 3v9d, 402d, 404d, 410d, 411d, 420d, 433d, 439d, 468d, 469d, 471d, 472d, 4e59, 4f4n, 4hqh, 4mcf, 4msb, 4nfo, 4nfp, 4nfq, 4o41, 4okl, 4u31, 4u3o, 4u3p, 4u3r, 4u78, 4xw0, 5axe, 5axf, 5ay3, 5ay4, 5der, 5dhb, 5e36, 5hbx, 5hn2, 5hnj, 5hnq, 5iye, 5iyg, 5iyj, 5krg, 5lqo, 5lqt, 5lr4, 5mvt, 5mvu, 5tgp, 5ued, 5uee, 5uef, 5v0h, 5v0j, 5v11, 5v2h, 5wv7, 5zas, 6bgb, 6c8d, 6c8e, 6c8i, 6c8j, 6c8k, 6c8m, 6c8n, 6c8o, 6cab, 6cxz, 6cy2, 6dy9, 6gn2, 6hu6-2, 6u6j, 6u89, 6u8u, 6vem, 6wy3, 6zpf, 6zr1, 6zrl, 6zrs, 6zw3, 6zwu, 6zx5, 6zx8, 7a9l, 7a9n, 7a9o, 7a9p, 7a9s, 7a9t, 7bpg, 7eck, 7ecm, 7eco, 7ecp, 7edu, 7kuk, 7kum, 7kun, 7kuo, 7kup, 7lne, 7lnf, 7qtn, 7u89, 7u8a, 7u8b, 8ami, 8amn, 8cry, 8swg, 8swo, 8vaw (200) | 101d, 102d, 109d, 111d, 112d, 113d, 126d, 127d, 130d, 144d, 158d, 166d, 182d, 1bdn, 1bna, 1cvy, 1cw9, 1d22, 1d27, 1d45, 1d46, 1d57, 1d63, 1d64, 1d65, 1d77, 1d85, 1d86, 1d98, 1dhh, 1dn9, 1dne, 1dnm, 1dou, 1dpn, 1edr, 1eel, 1eg6, 1ei4, 1em0, 1f69, 1fd5, 1fdg, 1fmq, 1fn1, 1fn2, 1fq2, 1ftd, 1hq7, 1i47, 1j8l, 1jgr, 1k2z, 1lex, 1ley, 1lp7, 1m6f, 1n1o, 1n4e, 1ply, 1qc1, 1qv8, 1rqy, 1s9b, 1sk5, 1vtj, 1vzk, 1xcs, 1xcu, 1z5t, 1z8v, 1zfb, 1zph, 1zpi, 208d, 218d, 224d, 233d, 245d, 250d, 258d, 261d, 263d, 264d, 265d, 266d, 267d, 268d, 269d, 270d, 271d, 287d, 289d, 290d, 291d, 296d, 298d, 2b0k, 2b3e, 2bna, 2d25, 2dbe, 2des, 2dp7, 2dpb, 2dpc, 2fih, 2fii, 2gvr, 2gyx, 2i2i, 2nlm, 2qef, 2qeg, 2rf3, 302d, 303d, 308d, 311d, 334d, 355d, 360d, 365d, 367d, 378d, 389d, 3ajk, 3ajl, 3bna, 3eil, 3ey3, 3fl6, 3gjh, 3gjj, 3gjl, 3i5l, 3n4n, 3opi, 3u05, 3u08, 3u0u, 407d, 408d, 443d, 444d, 445d, 446d, 447d, 448d, 454d, 455d, 456d, 457d, 458d, 459d, 463d, 473d, 474d, 476d, 477d, 478d, 4agz, 4ah0, 4ah1, 4bna, 4c5x, 4c64, 4d9x, 4dnb, 4f2x, 4f3u, 4gju, 4glh, 4hli, 4hw1, 4i9v, 4ij0, 4itd, 4kw0, 4l24, 4mnb, 4o5w, 4o5x, 4o5y, 4o5z, 4ocd, 4pwm, 4qc7, 4r49, 4r4a, 4u8c, 4z4b, 5bna, 5cjy, 5et9, 5ezf, 5fhj, 5i4s, 5ju4, 5kek, 5lj4, 5t4w, 5vy6, 5w20, 5xuv, 6adv, 6bna, 6qjr, 6udn, 6wr9, 6wra, 6wrc, 6wrj, 6wsp, 6wsw, 6wsz, 6x5d, 7bna, 7jki, 7jl9, 7jle, 7jog, 7jp8, 7jpb, 7js0, 7jsb, 7kci, 7ku4, 7kwk, 7rqt, 7zql, 8ce2, 8ec1, 8ed6, 8eda, 8edb, 8f1s, 8f1v, 8f20, 8f2w, 8f2y, 8fb4, 8fdq, 8fdr, 8ri3, 8ri5, 8t7x, 8ta8, 8ta9, 8tap, 8tb3, 8tbd, 8tbo, 8tc4, 8wnb, 8xp9, 9bna (250) |

| | | |
|---|---|---|
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**Table S2:** Summary of the best MR statistics, the RMSDs, and the statistics after the first round of refinement for all test cases.

| PDB ID | MR | | RMSD / Å | | | First Round of Refinement | |
|---|---|---|---|---|---|---|---|
| | LLG | TFZ | | | | R-factor / % | R-free / % |
| 257D | 223 | 11.8 | 0.658 | | | 21.9 | 28.9 |
| 1T0E | 343 | 15.3 | Long: 0.814 | Short: 0.776 | | 36.0 | 38.3 |
| 3TD0 | 390 | 13.1 | Long: 0.789 | Short: 0.917, 0.444 | | 42.3 | 44.6 |
| 3TD1 | 514 | 16.4 | Long: 0.534 | Short: 0.689, 0.750 | | 37.2 | 43.1 |
| 2TRA | 269 | 11.3 | AT duplex: 0.962 | D duplex: 0.626 | A duplex: 1.012 | 35.7 | 40.9 |

**Table S3:** The number of created models and the approximate total time taken for MR for each test case. The time is shown for both a standard computer and a high-performance computer. A standard computer features that the CPU is AMD Ryzen 5 7530U, the RAM is 16GB, and the storage is 512GB SSD. A high-performance computer features that the CPU is Intel Core i7-14700, the RAM is 64GB, and the storage is 500GB SSD and 1TB HDD.

| PDB ID | Stages of MR | No. of models | Time (Standard) | Time (High) |
|---|---|---|---|---|
| 257D | 1st round | 81 | 5 min | 3 min |
| | 2nd round | 36 | 2 min | < 1 min |
| 1T0E | 1st round for long stem | 243 | 76 hour | 28 hour |
| | 2nd round for long stem | 36 | 2.5 hour | 1 hour |
| | 1st round for short stem | 81 | 4 hour | 1.5 hour |
| | 2nd round for short stem | 27 | 1 hour | 30 min |
| 3TD0 | 1st round for long stem | 243 | 61.5 hour | 49 hour |
| | 2nd round for long stem | 48 | 9 hour | 5 hour |
| | 1st round for short stem | 27 | 5 hour | 3 hour |
| | 2nd round for short stem | 27 | 4 hour | 1.5 hour |
| 3TD1 | 1st round for long stem | 243 | 11.5 hour | 8 hour |
| | 2nd round for long stem | 27 | 1.5 hour | 1 hour |
| | 1st round for short stem | 27 | 1.5 hour | 1 hour |
| | 2nd round for short stem | 36 | 1.5 hour | 1 hour |
| 2TRA | 1st round for AT duplex | 2187 | 109 hour | 72 hour |
| | 2nd round for AT duplex | 54 | 1.5 hour | 1 hour |
| | 1st round for D duplex | 27 | 30 min | 20 min |
| | 2nd round for D duplex | 27 | 20 min | 10 min |
| | 1st round for A duplex | 147 | 25 min | 15 min |
| | 2nd round for A duplex | 45 | 10 min | 5 min |

**Table S4:** Crystal data, data-collection statistics and structure-refinement statistics for the newly solved structure deposited as PDB ID 25LU.

| Data | Values |
|---|---|
| **Crystal data** | |
| Space group | $P\,4_3$ |
| Unit cell (Å) | a=b=93.154, c=25.277 |
| No. of NA molecules in AU [a] | 4 |
| **Data collection** | |
| Beamline | BL-17A of Photon Factory |
| Wavelength (Å) | 0.98 |
| Resolution (Å) | 31.1-2.0 |
| of the outer shell (Å) | 2.1-2.0 |
| Unique reflections | 15171 |
| Completeness (%) | 99.9 |
| in the outer shell (%) | 100 |
| $R_{merge}$ [b] (%) | 5.5 |
| in the outer shell (%) | 32.8 |
| Redundancy | 6.6 |
| in the outer shell | 6.6 |
| $I/\sigma(I)$ | 20.0 |
| in the outer shell | 5.7 |
| CC(1/2) | 99.8 |
| in the outer shell | 95.0 |
| **Structure refinement** | |
| Resolution range (Å) | 31.1-2.0 |
| Used reflections | 15162 |
| $R$-factor [c] (%) | 21.7 |
| $R_{free}$ [d] (%) | 26.3 |
| R.m.s.d. bond length (Å) | 0.010 |
| R.m.s.d. bond angles (°) | 1.354 |

[a] Number of nucleic acid molecules in the asymmetric unit.

[b] $R_{merge} = 100 \times \Sigma_{hklj} |I_{hklj} - \langle I_{hklj} \rangle| / \Sigma_{hklj} \langle I_{hklj} \rangle$.

[c] $R$-factor = $100 \times \Sigma \, ||F_o| - |F_c|| / \Sigma \, |F_o|$, where $|F_o|$ and $|F_c|$ are optimally scaled observed and calculated structure factor amplitudes, respectively.

[d] Calculated using a random set containing 10% of observations.

**Supplemental methods**

## 1. Instructions

### 1.1. Installation

4MRNA can be installed by sequentially executing the following commands (Script S4) in a command-line interface (CLI) that can run shell scripts, such as Windows Subsystem for Linux (WSL) on Windows, Terminal on macOS, or a Linux terminal. In addition, the external programs *3DNA*, *Phenix*, and *CCP4* are required. These programs should be installed separately from their official websites according to the instructions provided there, and they should be executable from the command line.

**Script S4:** Installation commands of 4MRNA.

```
cd $HOME
git clone https://github.com/S-Ando-Biophysics/4MRNA-Install.git
bash 4MRNA-Install/install.sh
echo 'export PATH="$HOME/4MRNA-Install/bin:$PATH"' >> ~/.bashrc
source ~/.bashrc
```

### 1.2. Usage

First, enter a command in the CLI to move to the directory containing the reflection file (MTZ format). It is also recommended to create a new directory for 4MRNA and copy the reflection file into this directory. Next, enter "4MRNA" in the CLI. These steps are summarized in Script S5.

**Script S5:** Commands to start 4MRNA.

```
cd <path_to_directory_containing_the_reflection_file>
4MRNA
```

After 4MRNA is launched, the user is first asked how many types of models will be used for MR. This question corresponds to how many stem regions the target molecule should be divided into, as described in detail in the Tutorials section below. For each model, the user is then asked to enter three types of information: the nucleic acid type, selected from A-form DNA, B-form DNA, and A-form RNA; the sequence; and the number of copies to be searched for in the asymmetric unit (ASU) of the crystal. After all this information has been entered, the setup of the scripts running in the background of 4MRNA is completed.

Finally, the user selects either the default mode or the customize mode. In the default mode, all steps of 4MRNA are performed automatically, and the user only needs to wait for the results. This mode is recommended for all users. For expert users, the customize mode is also provided. When the customize mode is selected, the background scripts are downloaded and the program then terminates, allowing the user to edit the scripts manually and test more advanced options.

### 1.3. Checking the results

After 4MRNA has finished, a directory named "4MRNA-Results" is created. This directory contains three to seven MR results, which are automatically selected from the top-ranked solutions based on the MR statistics LLG and TFZ. These statistics are summarized in the file "4MRNA-results.txt".

The user should first check the statistical values listed in "4MRNA-results.txt". Based on our experience, for nucleic acid structures, solutions with LLG values greater than 100 and TFZ values greater than 8 may indicate a possible MR solution. Using these values as practical criteria, the user should visually inspect the agreement between the electron density map and the model, starting from the solutions with better statistics. If the model agrees well with the electron density map, the user can proceed to refinement. In some cases, multiple solutions may show good statistics exceeding the above criteria and reasonable agreement with the electron density map. In such cases, our recommendation is to select the solution with the highest TFZ value first. If multiple solutions have the same TFZ value, the solution with the highest LLG value among them should be chosen.

## 2. Tutorials

### 2.1. Basic example (PDB ID 257D)

In this section, PDB ID 257D is used as a basic example to demonstrate how to run 4MRNA. 4MRNA was performed according to the following steps. The commands and required inputs are summarized in Script S6.

1. Launch the CLI
2. Move to the directory containing the reflection file.
3. Execute the command "4MRNA".
4. Enter the requested information in the CLI. In this example, the model information is as follows.
    - The number of models for MR: Since the entire molecule forms one duplex (it is treated as one stem region), the number of models for MR is entered as 1.
    - Nucleic acid type: A-form DNA.
    - Sequence: GCCGGC. (Only one strand of the duplex needs to be entered.)
    - How many copies to be searched in MR: Based on the unit cell parameters and space group, the ASU was estimated to contain one molecule.
5. Select the default mode. (In general, the default mode is recommended.)

**Script S6:** Commands and required inputs for a basic example. (white: questions displayed in the CLI)

```
cd <path_to_directory_containing_the_reflection_file>
4MRNA
The number of models for molecular replacement [1,2,3]: 1
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-DNA
Sequence (one strand of duplex, 5'→3'): GCCGGC
How many copies of the model to be searched in molecular replacement: 1
Please choose execution mode. [default,customize]: default
```

After 4MRNA had finished, the results were checked in the following steps.

1. Open the file “4MRNA-Results.txt” in the directory “4MRNA-Results” (Figure S16 and S17).
2. Check the statistics. In this example, all selected solutions exceeded the practical criteria of LLG ≧ 100 and TFZ ≧ 8. Among these solutions, the highest TFZ value was 11.8. Among the solutions with this TFZ value, the highest LLG value was 223. Therefore, based on the MR statistics, the MR result labeled “3DNAAD-12-7-14” was judged to be the best solution.
3. Open the electron density map (MTZ format) and the model coordinate file (PDB format) contained in the directory labeled “phaser-3DNAAD-12-7-14” using a molecular modeling software such as *Coot* (Figure S18 and S19). Then, visually check whether the model agrees well with the electron density map.

Since the MR statistics were good and the model agreed well with the electron density map, MR using 4MRNA was considered successfully completed. The selected solution was then used for subsequent refinement by the preferred method.


**Figure S16:** Contents of the directory “4MRNA-Results”.

```
3DNAAD-14-5-14 254 11.7
3DNAAD-12-5-14 245 11.6
3DNAAD-12-7-14 223 11.8
3DNAAD-21-14-14 220 11.8
3DNAAD-14-14-14 241 11.7
```

**Figure S17:** Contents of the file “4MRNA-Results.txt”.

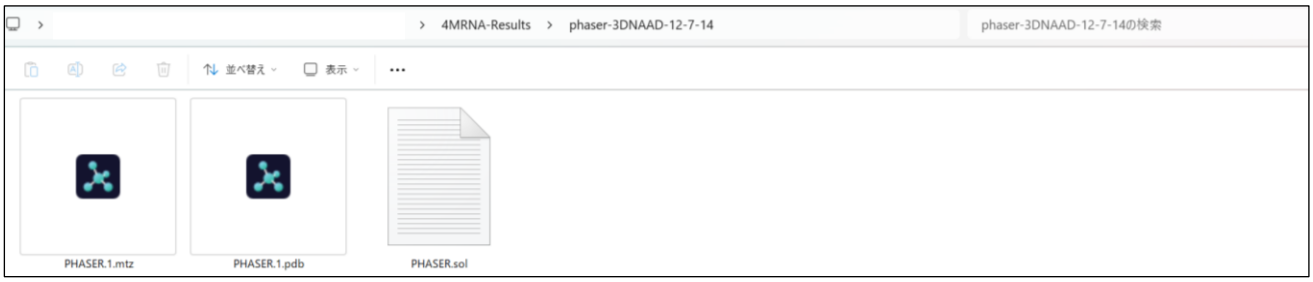


**Figure S18:** Contents of the directory “phaser-3DNAAD-12-7-14”.

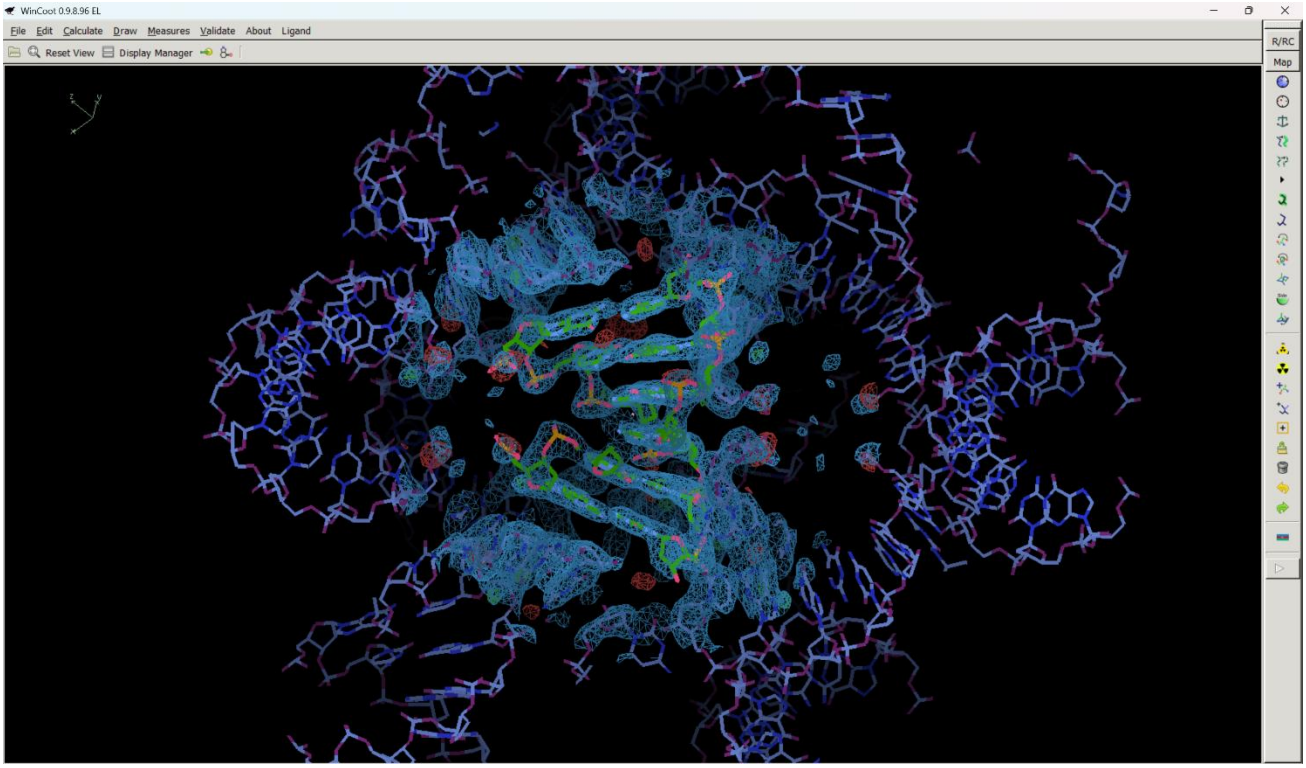


**Figure S19:** The electron density map and model opened in *Coot*.

### 2.2. Example using multiple models (PDB ID 3TD0)

In this section, PDB ID 3TD0 is used as an example to demonstrate how to enter the required information when multiple models are used.

This nucleic acid was designed so that the self-complementary sequence hybridizes to form double helical structure containing two internal loops, as shown in Figure S20. When the resulting duplex is considered from one side, it can be divided into a 5-bp stem, an internal loop, an 8-bp stem, an internal loop, and another 5-bp stem. The two terminal 5-bp stems have the same sequence because of self-complementarity. Therefore, in 4MRNA, MR is performed using two types of models: an 8-bp stem model and 5-bp stem models. In other words, the target molecule contains two types of stem regions. Accordingly, when asked for "The number of models for molecular replacement", the user should enter 2.

The information for the first model (8-bp stem model) is as follows.

- Nucleic acid type: A-form RNA.
- Sequence: CGUCGACG. (Only one strand of the duplex needs to be entered.)
- Number of copies to be searched for in MR: The ASU was estimated to contain one entire double-helical molecule based on the unit cell parameters and space group. Since the 8-bp stem occurs at one position in this molecule, the number of copies to be searched for is 1 × 1 = 1.

The information for the second model (5-bp stem model) is as follows.

- Nucleic acid type: A-form RNA.
- Sequence: GCGUC. (Only one strand of the duplex needs to be entered.)

- Number of copies to be searched for in MR: The ASU was estimated to contain one entire double-helical molecule based on the unit cell parameters and space group. Since the 5-bp stem occurs at two positions in this molecule, the number of copies to be searched for is 1 × 2 = 2.

After all required inputs had been provided, the default mode was selected to start the 4MRNA workflow. The procedures after completion of 4MRNA can be the same as those described in Tutorial 2.1.

The commands and required inputs are summarized in Script S7.

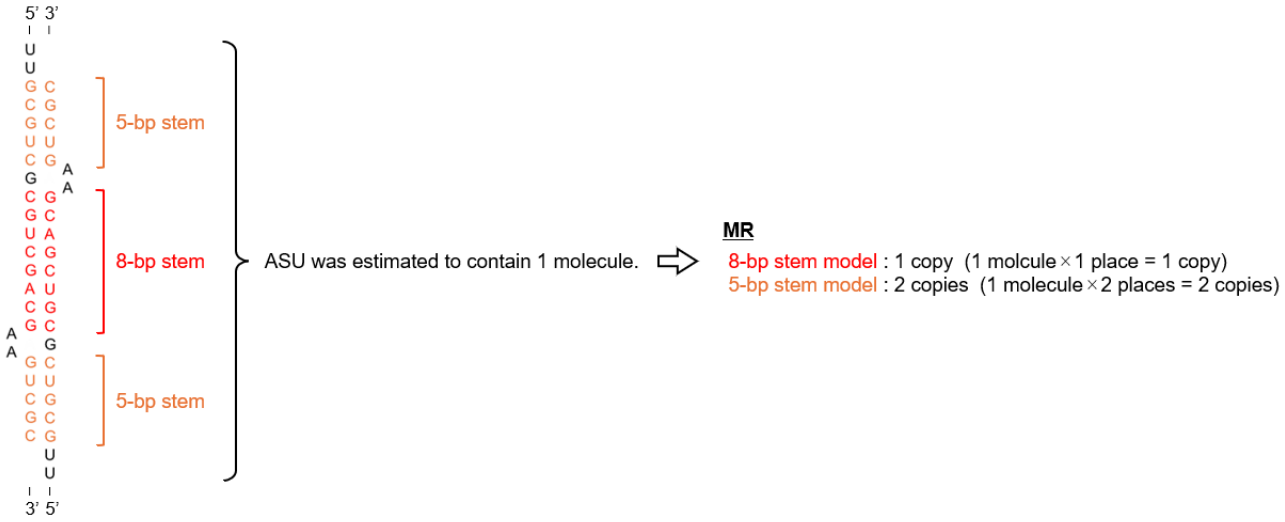


**Figure S20:** Sequence and the number of molecules in the asymmetric unit.

**Script S7:** Commands and required inputs for an example using multiple models.

```
cd <path_to_directory_containing_the_reflection_file>
4MRNA
The number of models for molecular replacement [1,2,3]: 2

Enter information of model #1.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): CGUCGACG
How many copies of the model to be searched in molecular replacement: 1

Enter information of model #2.
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-RNA
Sequence (one strand of duplex, 5'→3'): GCGUC
How many copies of the model to be searched in molecular replacement: 2

Please choose execution mode. [default,customize]: default
```

### 2.3. Example of a structure with only one strand in the asymmetric unit (PDB ID 118D)

In some cases, only one strand is present in the ASU, and this strand forms a double helix with a symmetry-related strand generated by crystallographic symmetry. In such cases, a single-stranded model should be prepared for MR. Although 4MRNA is basically designed to use duplex models composed of two strands, it also supports cases in which only one strand is present in the ASU. In this case, the user can enter 0.5 as the number of copies to be searched for in MR. This value "0.5" means half of one double-helical molecule, corresponding to one strand.

Here, PDB ID 118D (Bingman et al. 1992) is used as an example. Based on the unit cell parameters and space group, the ASU was estimated to contain one strand (0.5 of a duplex molecule). The commands and inputs in the CLI for this example are shown in Script S8. In addition, the best MR result displayed in *Coot* is shown in Figure S21. This figure shows that the strand in the ASU forms a duplex with a symmetry-related strand.

**Script S8:** Commands and required inputs for an example of a structure with only one strand in the ASU.

```
cd <path_to_directory_containing_the_reflection_file>
4MRNA
The number of models for molecular replacement [1,2,3]: 1
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-DNA
Sequence (one strand of duplex, 5'→3'): GTGCGCAC
How many copies of the model to be searched in molecular replacement: 0.5
Please choose execution mode. [default,customize]: default
```

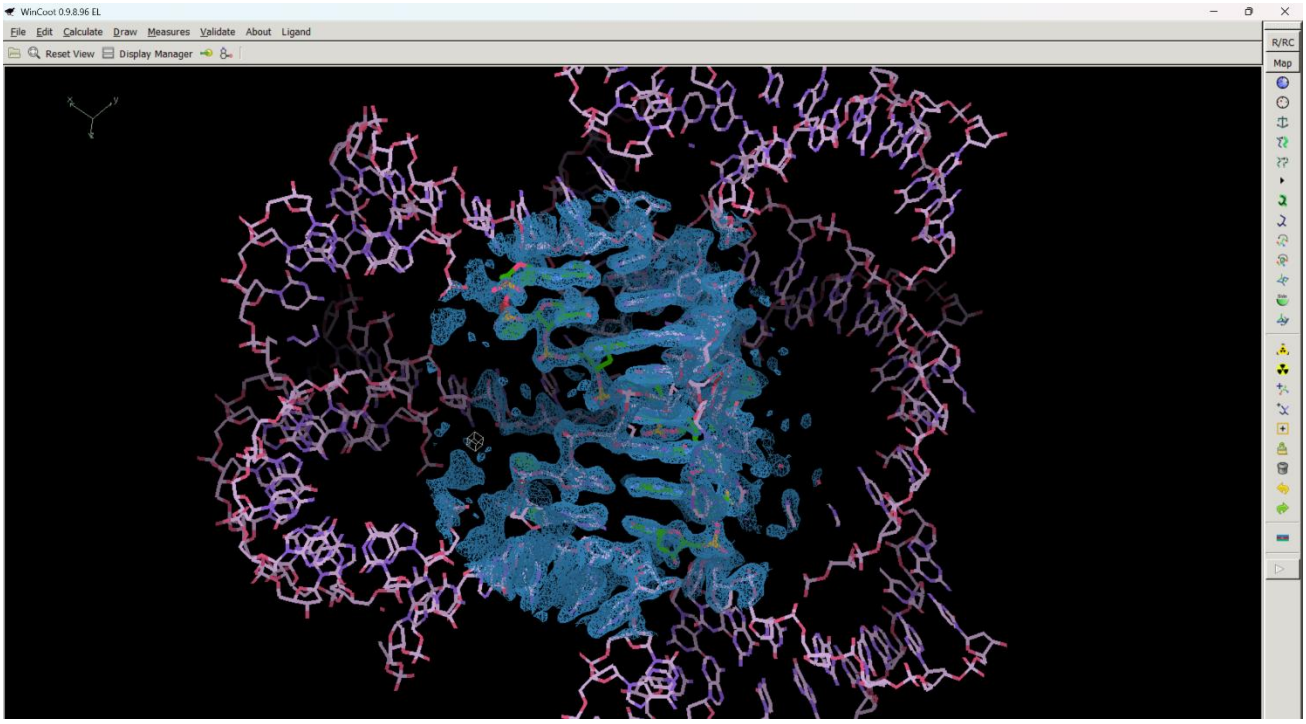


**Figure S21:** The best MR result opened in *Coot*. (Green: strand in the ASU; pink: symmetry-related strand)

### 2.4. Example of a structure with an odd number of strands in the asymmetric unit (PDB ID 5E36)

In some cases, an odd number of strands are present in the ASU. Some of these strands can form duplexes within the ASU, whereas the remaining strand can form a duplex with a symmetry-related strand generated by crystallographic symmetry. For example, when three strands are present in the ASU, two strands can form a duplex within the ASU, while the remaining one strand forms a duplex with a symmetry-related strand.

In such cases, the duplexes formed within the ASU should first be determined. The remaining strand can then be placed manually if the corresponding electron density is clearly visible. Alternatively, an additional MR search can be performed using a single-stranded model with the already determined duplexes fixed.

Here, PDB ID 5E36 (Wang et al. 2019) is used as an example. Based on the unit cell parameters and space group, the ASU was estimated to contain three strands (two duplex molecules and one strands). The commands and inputs in the CLI for this example are shown in Script S9.

**Script S9:** Commands and required inputs for an example of a structure with three strands in the ASU.

```
cd <path_to_directory_containing_the_reflection_file>
4MRNA
The number of models for molecular replacement [1,2,3]: 1
Nucleic acid type [A-DNA,B-DNA,A-RNA]: A-DNA
Sequence (one strand of duplex, 5'→3'): GTGTACAC
How many copies of the model to be searched in molecular replacement: 1
Please choose execution mode. [default,customize]: default
```

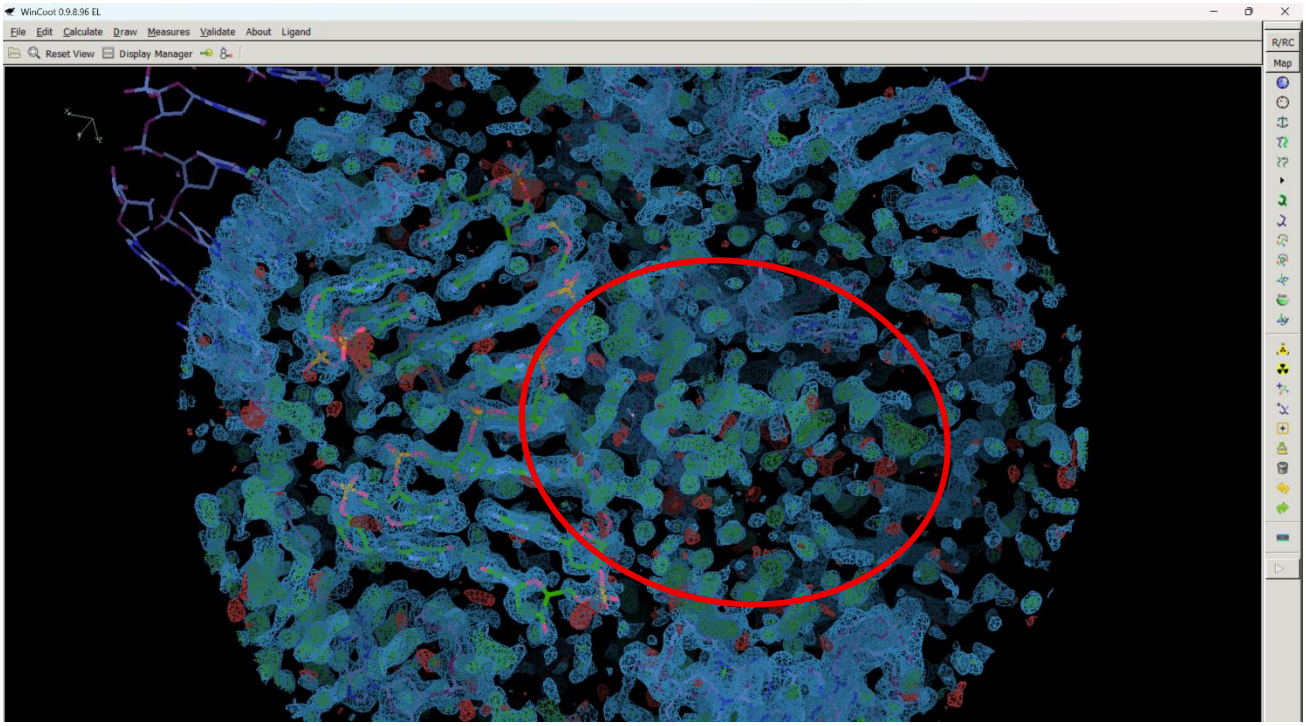


**Figure S22:** The best MR result obtained by 4MRNA opened in *Coot*.

The best MR result obtained by 4MRNA was displayed in *Coot*, as shown in Figure S22. This figure confirmed that the electron density corresponding to the unplaced strand was clearly visible. The enlarged view of this region is shown in Figure S23A. Since the third G was most clearly visible in the electron density map, this base was first placed manually (Figures S23B and S23C). Starting from this G, the surrounding residues were placed manually (Figure S23D).

After the remaining strand had been built and refinement was performed, the resulting model was obtained as shown in Figure S24. The remaining strand placed by manually forms a duplex with a symmetry-related strand.

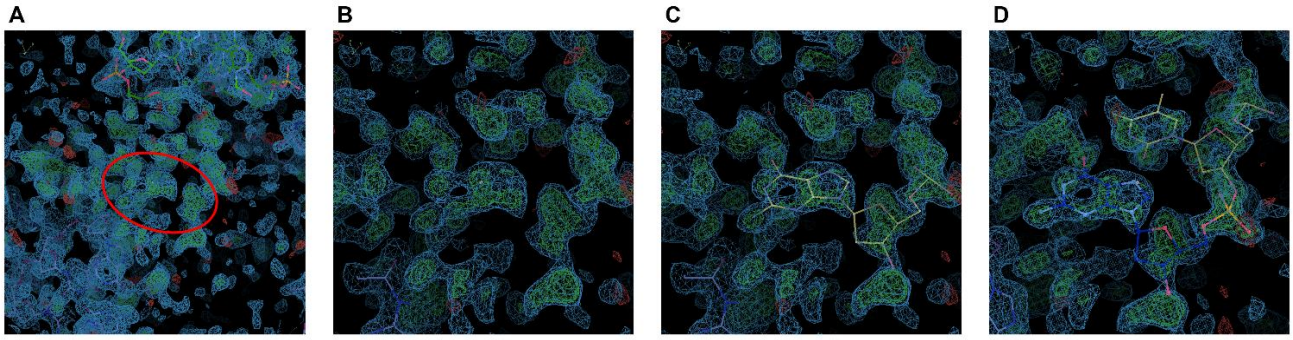


**Figure S23:** Manual modeling of the remaining strand based on the electron density map.

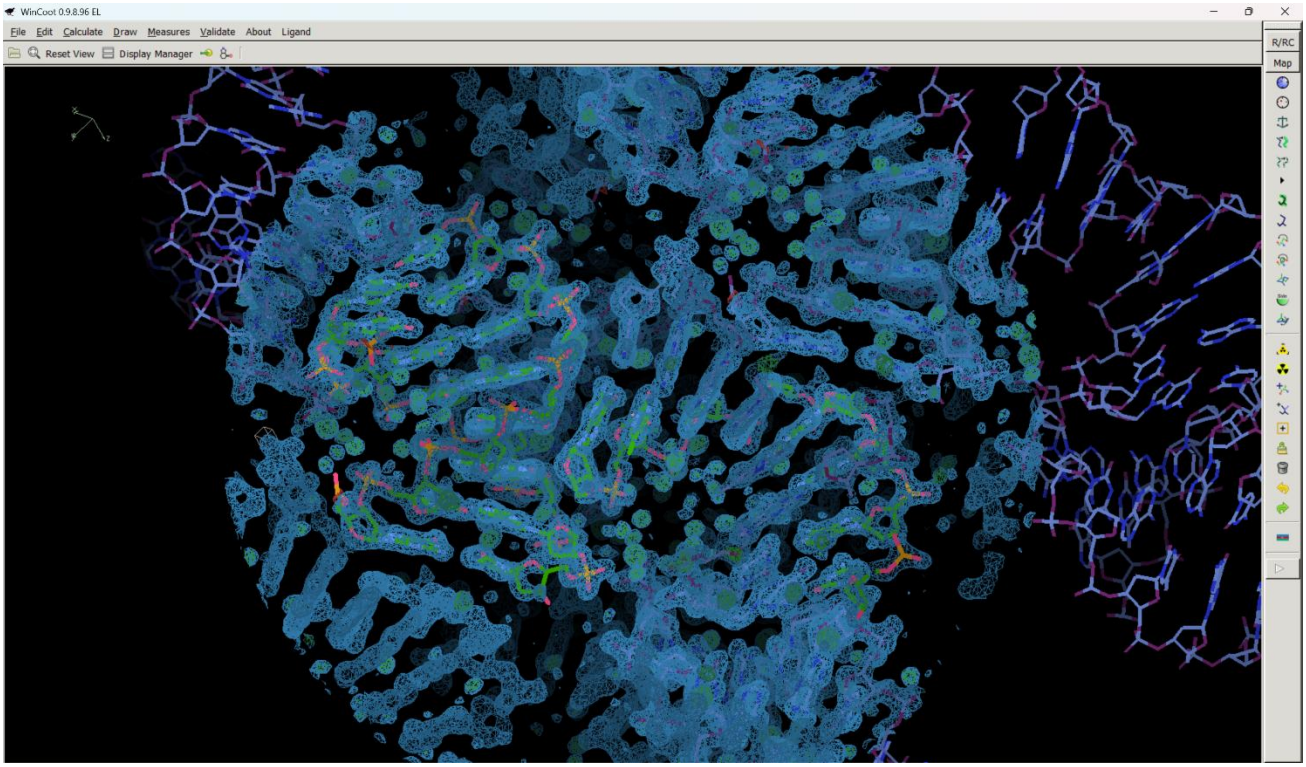


**Figure S24:** Refined model after manual modeling of the remaining strand.

**Supplemental references**

Bingman C, Li X, Zon G, Sundaralingam M. 1992. Crystal and molecular structure of d(GTGCGCAC): investigation of the effects of base sequence on the conformation of octamer duplexes. *Biochemistry* **31**: 12803-12812. doi:10.1021/bi00166a014

Wang R, Ranganathan SV, Haruehanroengra P, Mao S, Scalabrin M, Fabris D, Chen A, Liu H, Hassan AEA, Gan J, et al. 2019. Construction and structure studies of DNA-bipyridine complexes as versatile scaffolds for site-specific incorporation of metal ions into DNA. *J Biomol Struct Dyn* **37**: 551-561. doi:10.1080/07391102.2018.1441071