Enzyme Nanorings

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Supplemental Information

Fortran95 Script for Generating Bead Coordinates.

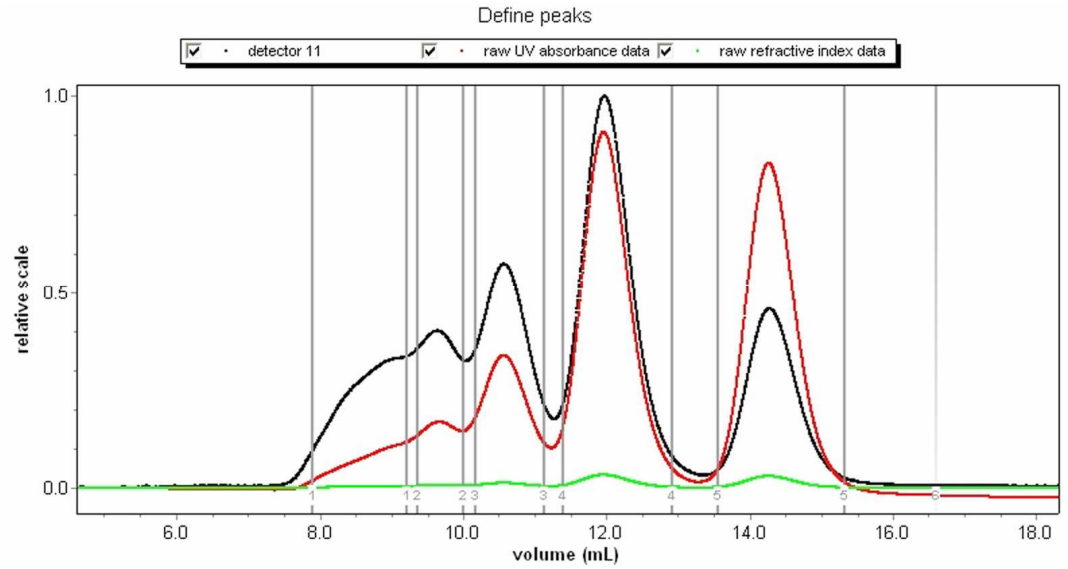
```
! This script calculates the positions of beads in a
! nanoring with differing radii (max 2 different beads)
! NOTE - the proteins can only bind heterodimerically (in even numbers)
PROGRAM DHpoly
IMPLICIT NONE
REAL :: radiusa, halfangle, xcora, ycora, zcora, radius
REAL :: radiusb, nsides, npoints, pi, xcorb, ycorb, zcorb
REAL :: xcorc, ycorc, zcorc, xcord, ycord, zcord, molwt, iter
INTEGER :: i, j, k, n
CHARACTER(len=21) :: datafile, inpfile
pi=3.141592654
zcora=0.0
zcorb=0.0
zcorc=0.0
zcord=0.0
WRITE(6,*) ''
WRITE(6,*) 'Welcome to the Wagner Lab heterodimeric nanoring coordinates
generator!'
WRITE(6,*) 'Enter the radius of protein 1 (nm):'
READ (5,*) radiusa
WRITE(6,*) 'Enter the radius of protein 2 (nm):'
READ (5,*) radiusb
WRITE(6,*) 'Enter the fusion protein molecular weight (kDa):'
READ (5,*) molwt
WRITE(6,*) 'This program generates nanorings up to the desired size in increments of 2.'
WRITE(6,*) 'Enter the maximum number of fusion proteins in the ring:'
READ (5,*) iter
WRITE(6,*) 'Thanks for playing "generate that nanoring"! Your HYDRO input files are
in'
WRITE(6,*) 'the same directory as this program. Have a nice day!'
N=iter
DO I=2,N,2
nsides=I
halfangle = pi/nsides
npoints = nsides*2-2
xcora=0
ycora=0
xcorb=0
ycorb=0
xcorc=0
ycorc=0
xcord=0
```

```
ycord=0
!write(*,*) 'number of sides =', nsides
if (I .LT. 9) then
write(datafile,2000)I,radiusa
write(inpfile,2001)I,radiusa
2000 format(I1,'gon',F4.2,'nm.dat')
2001 format(I1,'gon_inp',F4.2,'nm.dat')
end if
if (I .GT. 9) then
write(datafile,2002)I,radiusa
write(inpfile,2003)I,radiusa
2002 format(I2,'gon',F4.2,'nm.dat')
2003 format(I2,'gon_inp',F4.2,'nm.dat')
end if
OPEN (unit=10,file=datafile)
write(10,*) '1.E-07, !Unit of length, cm (10 A)'
write(10,"(I2,A20)") I*2,', !number of beads'
write(10,"(4F10.3)") xcora, ycora, zcora, radiusa
DO J=0, npoints, 4
xcorb=xcora+(radiusa+radiusb)*cos(halfangle*j)
ycorb=ycora+(radiusa+radiusb)*sin(halfangle*j)
write(10,"(4F10.3)") xcorb, ycorb, zcorb, radiusb
xcorc=xcorb+(radiusb+radiusb)*cos(halfangle*(j+1))
ycorc=ycorb+(radiusb+radiusb)*sin(halfangle*(j+1))
write(10,"(4F10.3)") xcorc, ycorc, zcorc, radiusb
xcord=xcorc+(radiusa+radiusb)*cos(halfangle*(j+2))
ycord=ycorc+(radiusa+radiusb)*sin(halfangle*(j+2))
write(10,"(4F10.3)") xcord, ycord, zcord, radiusa
xcora=xcord+(radiusa+radiusa)*cos(halfangle*(j+3))
ycora=ycord+(radiusa+radiusa)*sin(halfangle*(j+3))
if (J .LT. npoints-2) then
write(10,"(4F10.3)") xcora, ycora, zcora, radiusa
end if
END DO
OPEN (unit=11,file=inpfile)
write(11,"(A16)")'1DH Polygon'
!!this assigns the name of the output files
if (I .LT. 9) then
write(11,2004) I,radiusa
2004 format(I1,'gon',F4.2,'nm')
end if
if (I .GT. 9) then
write(11,2005) I,radiusa
2005 format(I2,'gon',F4.2,'nm')
end if
write(11,"(A17)")datafile !!this tells hydro to read in the right coor file
```

```
write(11,"(A2)")'-1'
write(11,"(A4)")'296.'
write(11,"(A5)")'0.010'
K=molwt*I*1000
write(11,"(I7)") K
write(11,"(A5)")'0.760'
write(11,"(A3)")'1.0'
write(11,"(A3)")'26,'
write(11,"(A6)")'1.5e+7,'
write(11,"(A3)")'30,'
write(11,"(A6)")'80.E-8'
write(11,"(A6)")'10000,'
write(11,"(A1)")'1'
write(11,"(A1)")'*'
END DO
END
```

Figure 1S.

Overlaid traces of 3DH-GLE (20 μM, 1 equiv Bis-MTX-C_9) from a 90°C angle static light scattering detector (black), a UV detector (red), and a raw reflective index (green) detector. Table below shows the average molecular masses of five fractions of the eluted samples by estimated static light scattering and reflective index detectors and the calculated molecular



Fraction	SLS result Molecular mass (kDa)	Oligomer (subunit)	Calculated molecular mass + C9 (kDa)
1	405.8±3.7	6	391.2
2	277.4±6.4	4	260.8
3	205.3±5.0	3	195.6
4	134.8±3.4	2	130.4
5	68.86±5.6	1	65.2

Figure 2S.

Superdex G75 Size Exclusion Elution Profiles of 3DH-GLE (2.5 μM) and 3DH-GLE (2.5 μM) mixed with Bis-MTX-C_9. 3DH-GLE (dark blue trace), 3DH-GLE with 0.2 equiv C9 (green trace), and 1 equiv C9 (red trace).

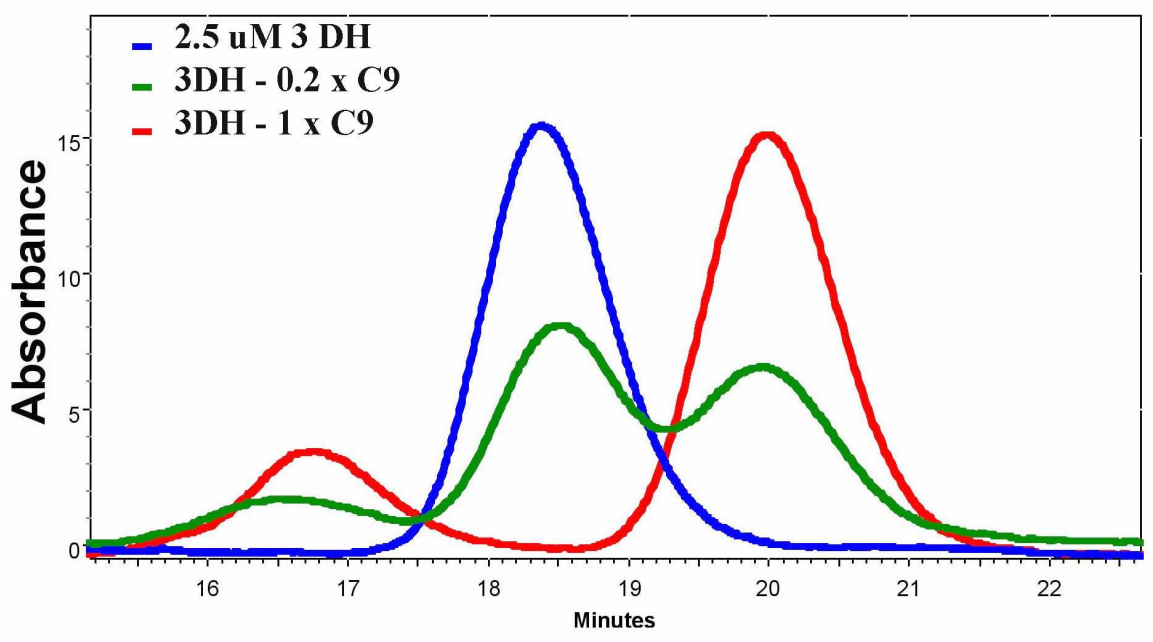
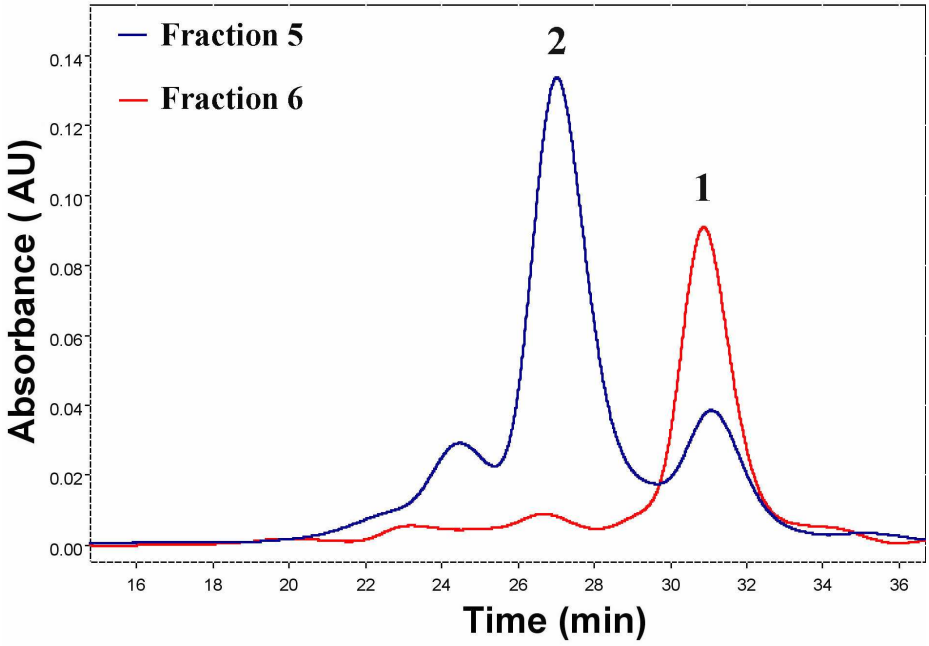
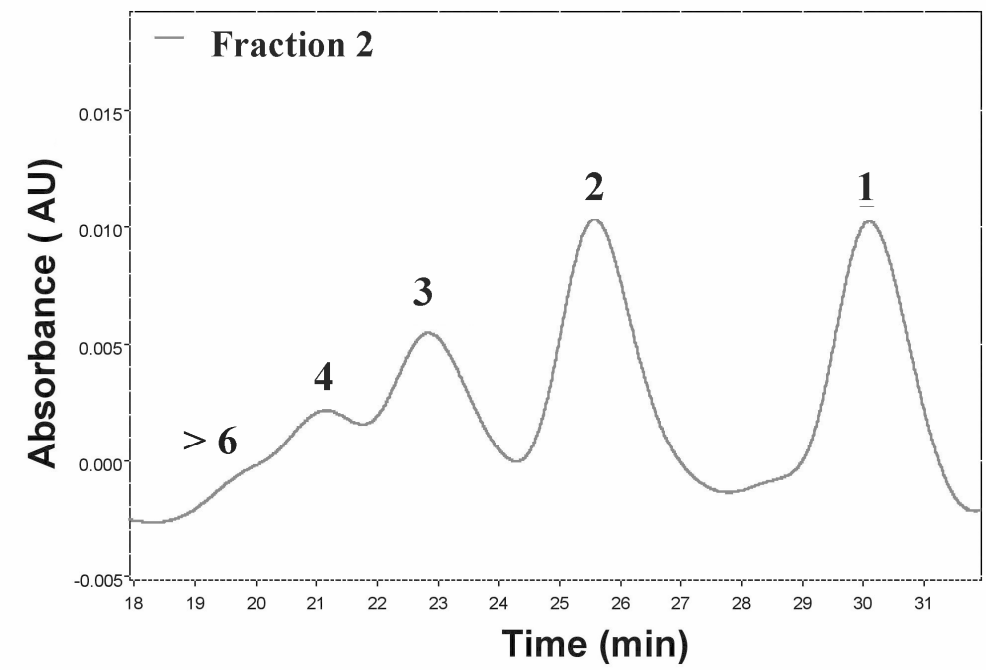


Figure 3S.

Stability of isolated 3DH-GLE-C_9 oligomeric fractions. Fractions 2, 5, and 6 were re-injected onto Supderdex G200. (A) the composition of fraction 5 and 6 remains unchanged after 3 months at 4 ^{o}C, whereas (B) the composition of the fraction 2 re-distributed into a range of oligomers.



A



B

Figure 4S.

4-12% SDS-PAGE of (A) purified DHFR-hHint1 fusion proteins. Lane1: 1DHG; Lane 2: 1DHT; Lane3 and Lane 6: 3DH-GLE; Lane 4: 7DH; Lane 5: 15DH; Lane 7: 3DH-GLG. The molecular weight of fusion proteins is around 32 kDa, which migrated similarly to the 30 kDa marker.

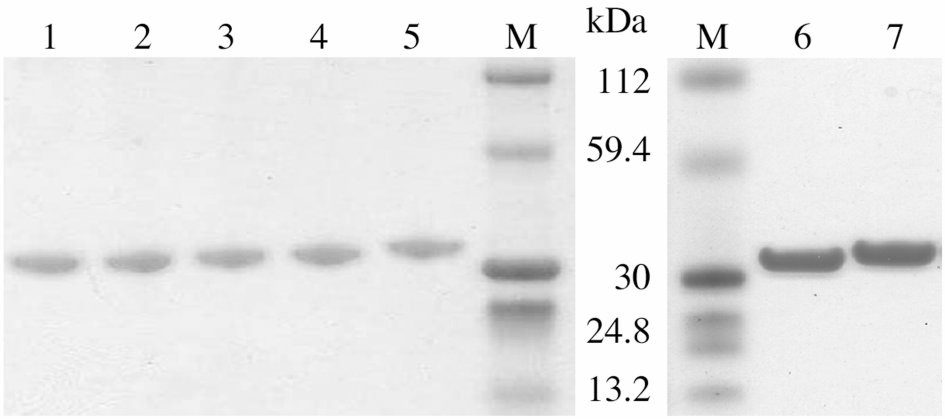
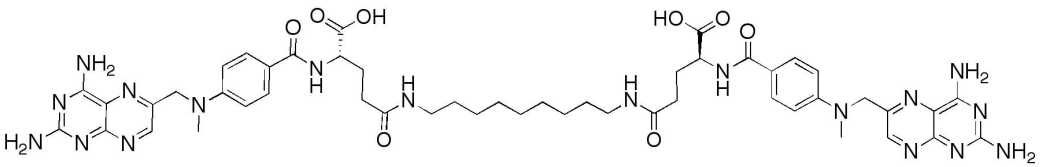


Figure 5S.

Structure of Bis-MTX-C_9



$C_{49}H_{62}N_{18}O_8$
Exact Mass: 1030.5
Mol. Wt.: 1031.13

Figure 6S.

SEC collected fraction displayed on mica, showing lowly dispersed rings of >200 nm diameter with no apparent rings of smaller dimension.

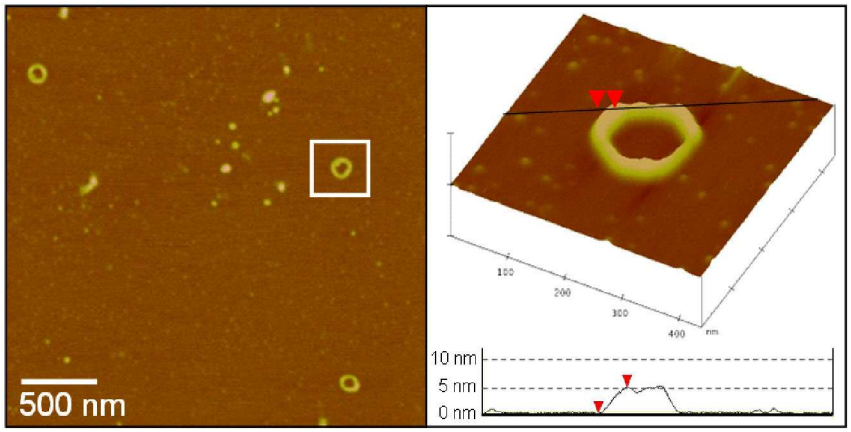


Figure 7S.

EDAX spectra of TS-Au surface, showing predominantly Au signal with no observed characteristic Si or Al signatures from residual mica left from sample processing

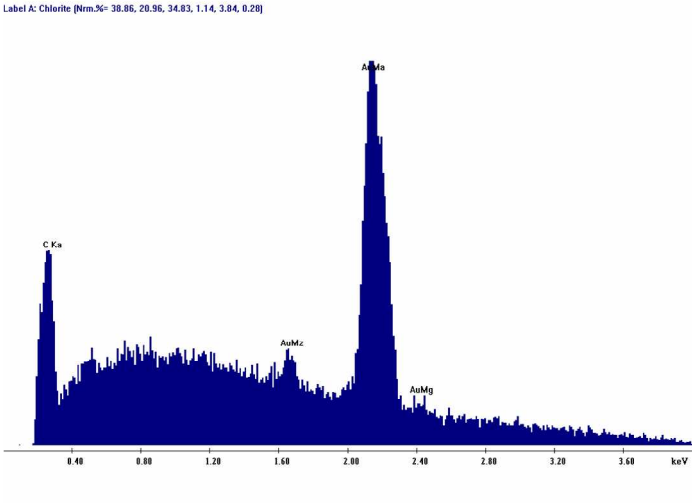


Table 1S. Primer sequences used in this paper.

Primer Name	Sequence
3DH-GLE-F	5′-GAGATTCTGGAGCGGCGGGGCCTCGAGATGGCAGATGAGATTGC-3′
3DH-GLE-R	5′-GCAATCTCATGTGCCATCTCGAGGCCCCGCCGCTCCAGAATCTC-3′
3DH-GLG-F	5′-GAGATTCTGGAGCGGCGGGGCCTCGGGATGGCAGATGAGATTGC-3′
3DH-GLG-R	5′-GCAATCTCATCTGCCATCCCGAGGCCCCGCCGCTCCAGAATCTC-3′
1DHG-F	5′-GAGATTCTGGAGCGGCGGGGCATGGCAGATGAGATTGC-3′
1DHG-R	5′-GCAATCTCATCTGCCATGCCCCGCCGCTCCAGAATCTC-3′
1DHT-F	5′-GAGATTCTGGAGCGGCGGACCATGGCAGATGAGATTGC-3′
1DHT-R	5′-GCAATCTCATCTGCCATGGTCCGCCGCTCCAGAATCTC-3′

Table 2S. Theoretical Molecular weight of DHFR-hHint1 fusion proteins.

Proteins	Calculated M. W. (Da)
1DHG	63680.64
1DHT	63768.74
3DH-GLE	63996.96
3DH-GLG	63906.96
7DH	64565.58
15DH	66209.54

Table 3S Hydrodynamic Parameters and Calculated Molecular Weights for DHFR-GLE-hHint from HYDRO++ Bead Modeling[a].

N[b]	R_s (nm)	R_g (nm)	R_g/R_s	Longest Axis (nm)	MW (kDa)
2	4.7	4.2	0.89	12.2	64
4	7.0	7.2	1.03	19.0	128
6	8.9	10.2	1.15	25.3	192
8	10.9	13.5	1.24	32.2	256
10	12.9	16.7	1.29	38.5	320
12	14.8	20.0	1.35	45.5	384
14	16.7	23.3	1.40	51.8	448
16	18.5	26.6	1.44	58.7	512
18	20.3	29.9	1.47	65.1	576
20	22.1	33.2	1.50	71.9	640
22	23.8	36.5	1.53	78.4	704
24	25.5	39.8	1.56	85.2	768
26	27.2	43.1	1.58	91.7	832
28	28.9	46.4	1.61	98.4	896
30	30.6	49.7	1.62	104.9	960

a DHFR bead radius of 2.85 nm and hHint bead radius of 2.35 nm were determined by measuring the protein dimensions in VMD and splitting the GLE linker radius (0.7 nm) between the two protein beads. **b** The value N equals the number of DHFR-mono(Hint1) subunits.