Supplementary Material (ESI) for Soft Matter
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SUPPORTING ONLINE MATERIAL

Nanocompartments with a pH-Controlled Release System based on an Engineered OmpF Channel Protein

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Experimental procedures

Primers

R42H_fwd	GGCGACATGACCTATGCCC**A**TCTTGGTTTTAAAGGGGAA
R42H_rev	TTCCCCTTTAAAACCAAGA**T**GGGCATAGGTCATGTCGCC
R82H_fwd	GACGCTCAAACTGGTAACAAAACGC**A**TCTGGCATTCGCGGGTCTTAAATAC
R82H_rev	GTATTTAAGACCCGCGAATGCCAGA**T**GCGTTTTGTTACCAGTTTGAGCGTC
R132H_fwd	AGCGATGACTTCTTCGTTGGTC**AC**GTTGGCGGCGTTGCTACCTAT
R132H_rev	ATAGGTAGCAACGCCGCCAAC**GT**GACCAACGAAGAAGTCATCGCT
D113H_fwd	GATGCACTGGGTTACACC**C**ATATGCTGCCAGAATTTGGTG
D113H_rev	CACCAAATTCTGGCAGCATAT**G**GGTGTAACCCAGTGCATC
E117H_fwd	GTTACACCCATATGCTGCCA**C**A**C**TTTGGTGGTGATACTGC
E117H_rev	GCAGTATCACCACCAAA**G**T**G**TGGCAGCATATGGGTGTAAC
D121H_fwd	CTGCCACACTTTGGTGGT**C**ATACTGCATACAGCGATGAC
D121H_rev	GTCATCGCTGTATGCAGTAT**G**ACCACCAAAGTGTGGCAG

Suppl. Table 1: Primers used in SDM to generate OmpF 6His mutant. The two-stage QuikChange derived SDM protocol was used with a first step of 3 cycles with 30 s denaturation at 95°C, 60 s annealing at 55°C and 8 min elongation at 68°C, followed by a second step with 15 cycles under the same conditions and a final elongation step of 20 min at 68°C.

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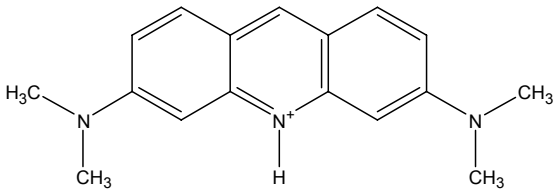
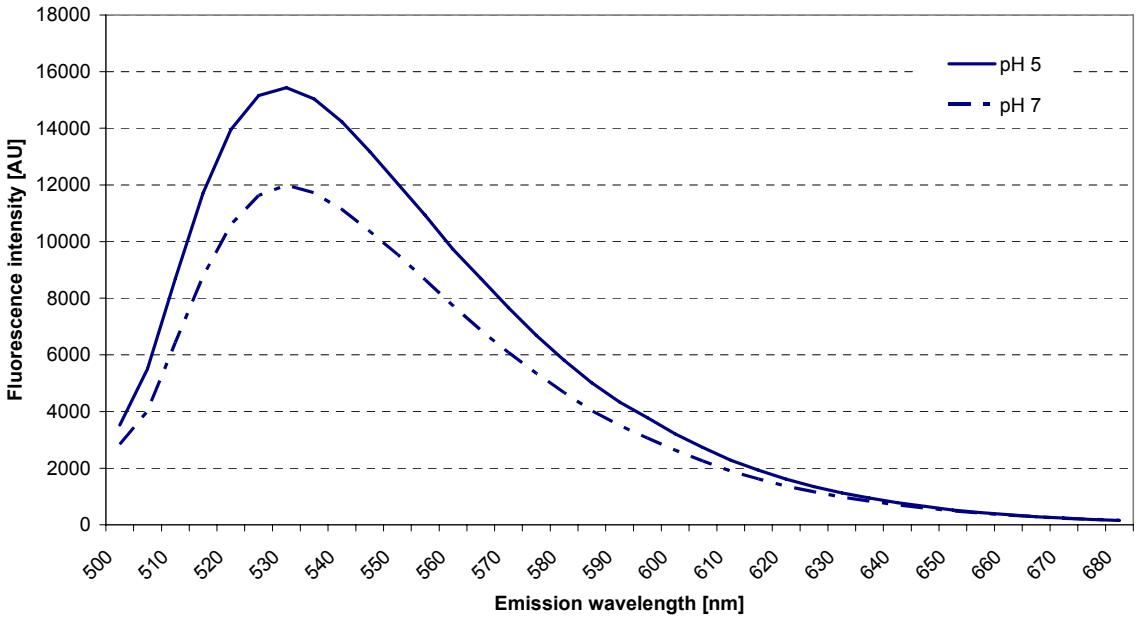
Acridine Orange

Acridine orange (Suppl. Figure 1) was selected as a model fluorophore in the experiments because of its charge and fluorescent characteristics. The dye is widely used in fluorescence microscopy for detection of DNA or RNA. Within the range of 5 ≤ pH ≤ 7 the dye is positively charged[1]. Acridine orange can form dimers[1] in the concentration range >10^{-5} M and <10^{-3} M with a pH ≤ 4. Because of the conditions used in the experiments, concentrations of 100 µM (see Nanocompartments Preparation section) coupled with a pH within 5 and 7, acridine orange is mostly in its monomeric state.

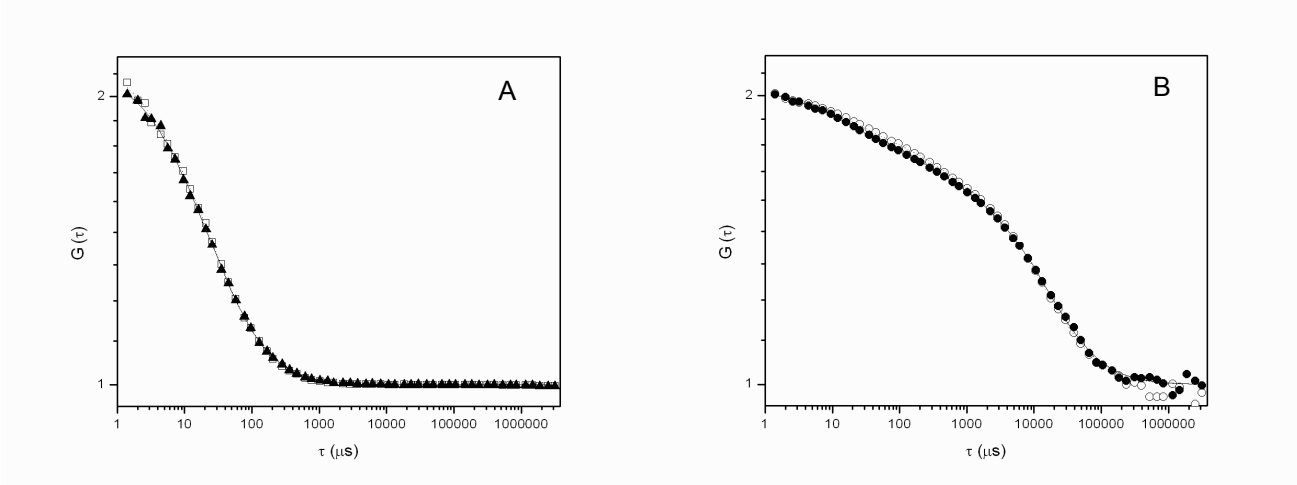


Suppl. Figure 1: Acridine orange structure.



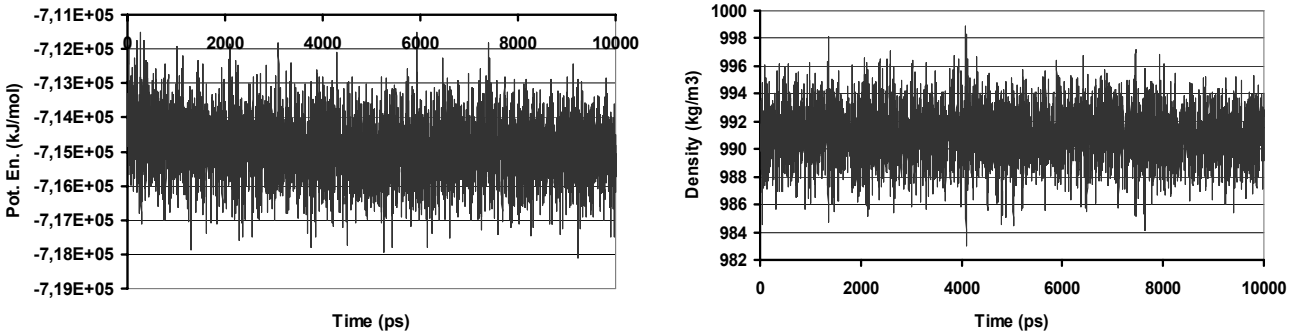
Suppl. Figure 2: Acridine orange emission spectra were recorded at λ_{Ex} = 490 nm for 30 µM acridine orange in K_xPO_4 buffer (50 mM, pH 5 (full line) and 7 (dashed line)). At this concentration and pHs the dye is a monomer[1].

FCS autocorrelation curves

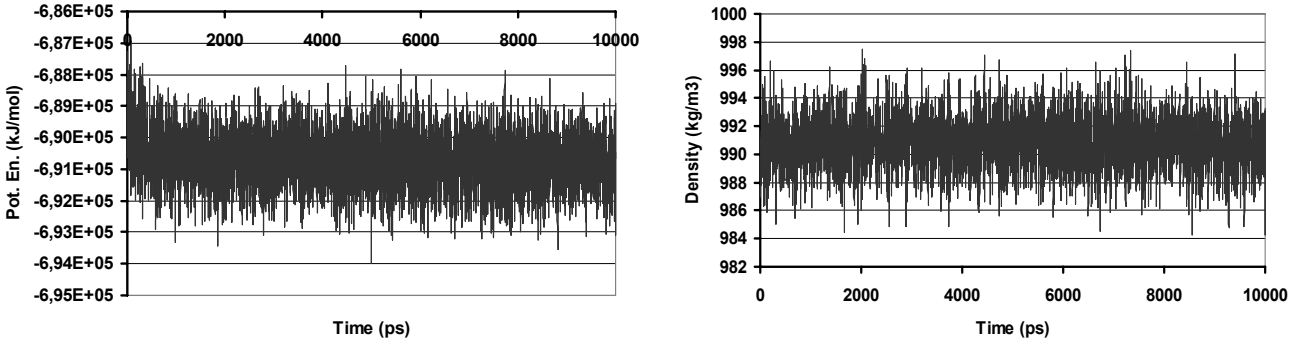


Suppl. Figure 3. The pH effect on both acridine orange and nanocopartments with reconstituted OmpF are negligible. (A) Autocorrelation curve of acridine orange measured at pH 5 (open squares) and at pH 7 (closed triangles). (B) Autocorrelation curves of polymeric nanocontainers with encapsulated acridine orange without membrane proteins at pH 5 (open circles) and pH 7 (closed circles).

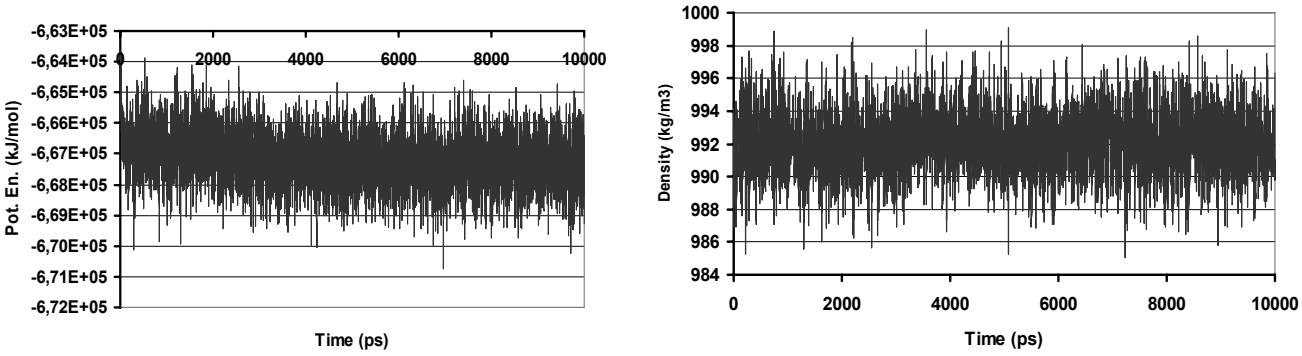
Molecular Dynamics Analysis



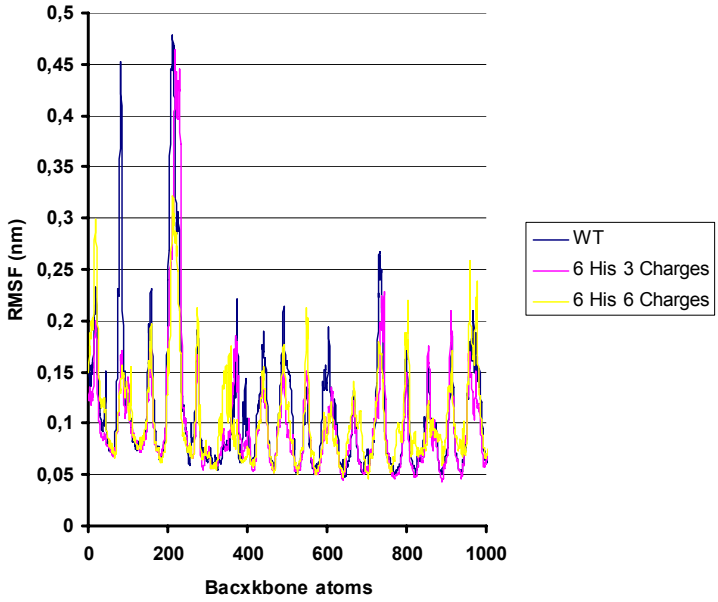
Suppl. Figure 4. Potential energy (σ=887, Tot-drift=-34) and density plots (σ =1.92, Tot-drift=-0.034) toward time for the OmpF WT protein. The system is well equilibrated within the last 5 ns.



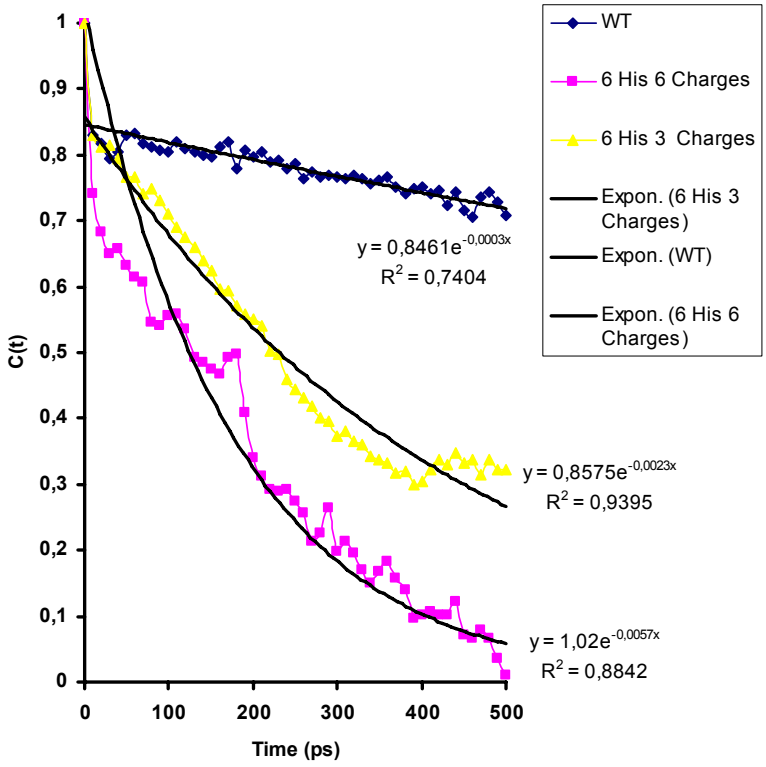
Suppl. Figure 5. Potential energy (σ=881, Tot-drift=-61) and density plots (σ=1.92, Tot-drift=-0.053) toward time for the OmpF 6His pH=5.0 protein. The system is well equilibrated within the last 5 ns.



Suppl. Figure 6. Potential energy (σ=862, Tot-drift=-76) and density plots (σ=2.0, Tot-drift=-0.07) toward time for the OmpF 6 His pH=7.0 protein. The system is well equilibrated within the last 5 ns.



Suppl. Figure 7. RMSF of the backbone atoms calculated on the last 5 ns of the trajectory. A clear difference between β-barrel regions (lowest fluctuations) and loops (higher fluctuations) is shown. The reported behavior is typical for β-barrel proteins as reported also in other MD simulations[2].



Suppl. Figure 8. Positional correlation time ($C_{(t)}$) of the Center of Mass (CM) of His 113 and the CM of His 42, 82 and 132. The C(t) function has been calculated by the relation: $C_t^{ij} = \frac{d_t^{ij} - d_\infty^{ij}}{d_0^{ij} - d_\infty^{ij}}$ where d^{ij} is the distance between the CM of His 113 and the CM of His 42, 82 and 132. The rapid decay of the charged His variants are within the 10 ns of simulation. (WT = 6666 ps, 6His (+6) = 350 ps, 6His (+3) = 868 ps)

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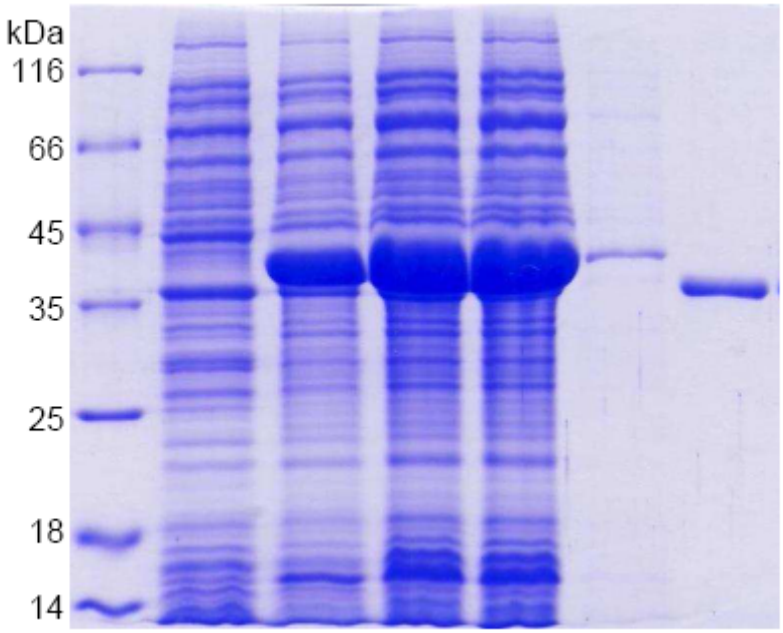
Protein Expression



Suppl. Figure 9. OmpF wildtype expression and extraction. Lane 1: Unstained Protein Molecular Weight Marker (Fermentas, St. Leon-Rot, Germany), lane 2: before induction, lane 3: end of fermentation, lane 4: after SDS lysis, lane 5: supernatant after 1st centrifugation, lane 6: supernatant after pre-extraction, lane 7: extracted OmpF.

Protein Quantification in Polymersome

An upper limit to the amount of protein measured in 10 ml polymersome solution has been set by the detection limit of the Coomassie staining (~50 ng), carried out after SDS PAGE. No protein band was reported after staining.

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CD Fitting Results

OmpF 6 His pH=5

0PLOT OF DATA (O) AND FIT TO DATA (X). ORDINATES LISTED ARE FIT VALUES.

```
   ORDINATE  ABSCISSA
 -1.545E+03  2.40E+02                          OX
 -1.737E+03  2.39E+02                          O X
 -2.129E+03  2.38E+02                          OX
 -2.631E+03  2.37E+02                         OX
 -3.139E+03  2.36E+02                         *
 -3.565E+03  2.35E+02                         *
 -3.881E+03  2.34E+02                        XO
 -4.155E+03  2.33E+02                        X O
 -4.410E+03  2.32E+02                       XO
 -4.749E+03  2.31E+02                       *
 -5.322E+03  2.30E+02                      OX
 -6.153E+03  2.29E+02                     *
 -7.053E+03  2.28E+02                    *
 -7.958E+03  2.27E+02                  XO
 -8.833E+03  2.26E+02                 *
 -9.705E+03  2.25E+02                *
 -1.059E+04  2.24E+02               *
 -1.147E+04  2.23E+02             XO
 -1.221E+04  2.22E+02            *
 -1.282E+04  2.21E+02           *
 -1.342E+04  2.20E+02          *
 -1.393E+04  2.19E+02         *
 -1.432E+04  2.18E+02XO
 -1.449E+04  2.17E+02*
 -1.446E+04  2.16E+02*
 -1.426E+04  2.15E+02OX
 -1.396E+04  2.14E+02 OX
 -1.351E+04  2.13E+02  OX
 -1.297E+04  2.12E+02    OX
 -1.232E+04  2.11E+02      *
 -1.146E+04  2.10E+02       XO
 -1.048E+04  2.09E+02         XO
 -9.241E+03  2.08E+02           X O
 -7.612E+03  2.07E+02              XO
 -5.536E+03  2.06E+02                 OX
 -3.276E+03  2.05E+02                    OX
 -7.938E+02  2.04E+02                       OX
  1.845E+03  2.03E+02                          *
  4.913E+03  2.02E+02                             *
  7.988E+03  2.01E+02                                *
  1.122E+04  2.00E+02                                   OX
  1.405E+04  1.99E+02                                      *
  1.642E+04  1.98E+02                                         *
  1.824E+04  1.97E+02                                            *
  1.970E+04  1.96E+02                                               *
  2.009E+04  1.95E+02                                               *
  2.016E+04  1.94E+02                                               *
  1.968E+04  1.93E+02                                               *
  1.888E+04  1.92E+02                                            *
  1.770E+04  1.91E+02                                         OX
  1.615E+04  1.90E+02                                      *
  1.035E+00  0.00E+00                          *
1CONTIN VERSION 2DP (MAR 1984) ( CD-1  PACKAGE)  ++++++++++++++++++++++++++++++++  CHOSEN SOLUTION
++++++++++++++++++++++++++++++++

        TEST DATA SET 1 - FOR CD PACKAGE

     ALPHA    ALPHA/S(1)     OBJ. FCTN.       VARIANCE      STD. DEV.    DEG FREEDOM    PROB1 TO REJECT    PROB2
TO REJECT
  1.15E-04     2.84E-03    1.19886E+02    5.10347E+01      1.127E+00        11.834           0.719           0.997

   ORDINATE    ERROR  ABSCISSA
```

```
 1.569E-01  5.4E-02  1.00E+00                                   ...X...
-3.777E-01  9.6E-02  2.00E+00              .....X.....
 4.129E-01  6.2E-02  3.00E+00                                            ...X...
-2.985E-01  5.9E-02  4.00E+00                    ...X....
 5.171E-01  6.5E-02  5.00E+00                                             ....X...
-5.120E-01  8.1E-02  6.00E+00          ....X....
 4.734E-01  4.5E-02  7.00E+00                                             ..X...
-8.522E-01  7.2E-02  8.00E+00X...
-5.118E-01  9.9E-02  9.00E+00          .....X.....
 2.134E-01  1.1E-01  1.00E+01                                   ......X......
-5.031E-01  6.0E-02  1.10E+01           ...X....
-6.886E-02  5.6E-02  1.20E+01                         ...X...
 9.972E-01  5.3E-02  1.30E+01                                                  ..X
 8.385E-01  8.2E-02  1.40E+01                                               .....X....
 1.605E-01  9.3E-02  1.50E+01                                   .....X.....
 3.895E-01  6.0E-02  1.60E+01                                            ....X...
```

	HELIX	BETA-SHEET	REMAINDER	SCALE FACTOR
FRACTION	0.34	0.50	0.16	1.035
STANDARD ERROR	1.5E-02	2.7E-02	2.9E-02	

OmpF 6 His pH=7

0PLOT OF DATA (O) AND FIT TO DATA (X). ORDINATES LISTED ARE FIT VALUES.

```
  ORDINATE  ABSCISSA
-1.492E+02  2.40E+02                                *
-2.336E+02  2.39E+02                                OX
-3.673E+02  2.38E+02                                *
-5.859E+02  2.37E+02                                *
-9.361E+02  2.36E+02                                *
-1.460E+03  2.35E+02                                *
-2.073E+03  2.34E+02                                XO
-2.795E+03  2.33E+02                                XO
-3.497E+03  2.32E+02                                XO
-4.294E+03  2.31E+02                                *
-5.100E+03  2.30E+02                                OX
-6.006E+03  2.29E+02                                *
-6.919E+03  2.28E+02                                *
-7.841E+03  2.27E+02                                *
-8.694E+03  2.26E+02                                *
-9.591E+03  2.25E+02                                *
-1.041E+04  2.24E+02                                *
-1.120E+04  2.23E+02                                XO
-1.188E+04  2.22E+02                                XO
-1.240E+04  2.21E+02                                XO
-1.293E+04  2.20E+02                                *
-1.351E+04  2.19E+02                                *
-1.417E+04  2.18E+02                                *
-1.478E+04  2.17E+02                                *
-1.524E+04  2.16E+02                                *
-1.554E+04  2.15E+02                                *
-1.574E+04  2.14E+02                                *
-1.583E+04  2.13E+02                                *
-1.573E+04  2.12E+02                                *
-1.557E+04  2.11E+02                                *
-1.539E+04  2.10E+02                                *
-1.512E+04  2.09E+02                                *
-1.465E+04  2.08E+02                                XO
-1.377E+04  2.07E+02                                XO
-1.246E+04  2.06E+02                                OX
-1.121E+04  2.05E+02                                *
-9.513E+03  2.04E+02                                *
-7.594E+03  2.03E+02                                *
-5.208E+03  2.02E+02                                XO
-2.424E+03  2.01E+02                                *
 6.312E+02  2.00E+02                                *
 3.554E+03  1.99E+02                                *
 6.471E+03  1.98E+02                                XO
 9.168E+03  1.97E+02                                *
 1.150E+04  1.96E+02                                *
 1.321E+04  1.95E+02                                *
 1.430E+04  1.94E+02                                *
 1.498E+04  1.93E+02                                *
 1.508E+04  1.92E+02                                *
```

```
  1.495E+04  1.91E+02                                                                *
  1.478E+04  1.90E+02                                                                *
  1.022E+00  0.00E+00                                  *
1CONTIN VERSION 2DP (MAR 1984) ( CD-1  PACKAGE)  ++++++++++++++++++++++++++++++++  CHOSEN SOLUTION
++++++++++++++++++++++++++++++++

        TEST DATA SET 1 - FOR CD PACKAGE

    ALPHA    ALPHA/S(1)     OBJ. FCTN.       VARIANCE      STD. DEV.    DEG FREEDOM    PROB1 TO REJECT    PROB2 TO REJECT
  4.51E-05     1.11E-03   8.53819E+01    5.09879E+01     1.156E+00        13.825           0.025              0.937

   ORDINATE    ERROR  ABSCISSA
  2.464E-02  9.7E-02  1.00E+00                                  .....X....
 -1.067E-01  1.3E-01  2.00E+00                                .......X......
  6.816E-01  1.5E-01  3.00E+00                                                .......X.......
  6.500E-01  6.5E-02  4.00E+00                                                ...X....
 -4.304E-01  1.9E-01  5.00E+00                        .........X.........
  1.436E-01  8.0E-02  6.00E+00                                     ...X....
  4.189E-01  6.8E-02  7.00E+00                                          ....X...
 -1.149E+00  9.7E-02  8.00E+00.....X.....
 -1.264E+00  1.2E-01  9.00E+00X.....
 -3.551E-01  1.9E-01  1.00E+01                        .........X.........
  4.047E-01  6.1E-02  1.10E+01                                          ...X...
 -1.445E-01  6.7E-02  1.20E+01                                   ...X...
  3.811E-01  6.2E-02  1.30E+01                                          ...X...
  8.393E-01  9.8E-02  1.40E+01                                                   ....X
  3.127E-01  1.2E-01  1.50E+01                                      .....X......
  6.154E-01  1.4E-01  1.60E+01                                                .......X.......

                HELIX   BETA-SHEET    REMAINDER            SCALE FACTOR
 FRACTION          0.33        0.52        0.15                1.022
 STANDARD ERROR     2.1E-02     3.1E-02     4.0E-02
```

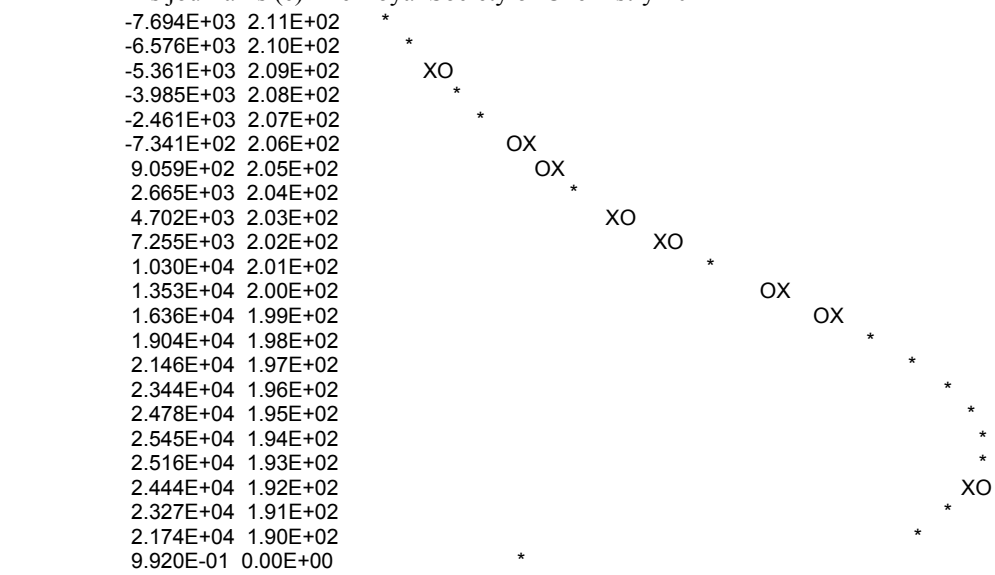
OmpF WT pH=5

```
0PLOT OF DATA (O) AND FIT TO DATA (X).  ORDINATES LISTED ARE FIT VALUES.

   ORDINATE  ABSCISSA
 -6.598E+02  2.40E+02                    OX
 -6.843E+02  2.39E+02                    OX
 -8.806E+02  2.38E+02                    OX
 -1.180E+03  2.37E+02                    *
 -1.501E+03  2.36E+02                    *
 -1.780E+03  2.35E+02                    *
 -2.021E+03  2.34E+02                    *
 -2.249E+03  2.33E+02                    *
 -2.476E+03  2.32E+02                    *
 -2.721E+03  2.31E+02                    *
 -3.067E+03  2.30E+02                   OX
 -3.525E+03  2.29E+02                   *
 -4.002E+03  2.28E+02                  *
 -4.493E+03  2.27E+02                  *
 -4.988E+03  2.26E+02                 *
 -5.498E+03  2.25E+02                *
 -6.046E+03  2.24E+02               *
 -6.590E+03  2.23E+02              *
 -7.067E+03  2.22E+02             XO
 -7.473E+03  2.21E+02            XO
 -7.868E+03  2.20E+02            *
 -8.180E+03  2.19E+02OX
 -8.376E+03  2.18E+02*
 -8.409E+03  2.17E+02*
 -8.299E+03  2.16E+02*
 -8.046E+03  2.15E+02 *
 -7.638E+03  2.14E+02  OX
 -7.040E+03  2.13E+02    *
 -6.234E+03  2.12E+02      *
 -5.187E+03  2.11E+02         XO
 -3.914E+03  2.10E+02            XO
 -2.446E+03  2.09E+02                *
 -7.547E+02  2.08E+02                    *
  1.090E+03  2.07E+02                        OX
  3.148E+03  2.06E+02                            OX
  5.132E+03  2.05E+02                                *
  7.326E+03  2.04E+02                                    *
  9.570E+03  2.03E+02                                        *
  1.201E+04  2.02E+02                                            *
```

```
 1.437E+04  2.01E+02                                                *
 1.655E+04  2.00E+02                                                  OX
 1.802E+04  1.99E+02                                                    *
 1.910E+04  1.98E+02                                                     *
 1.953E+04  1.97E+02                                                      *
 1.960E+04  1.96E+02                                                      *
 1.901E+04  1.95E+02                                                     *
 1.803E+04  1.94E+02                                                    *
 1.669E+04  1.93E+02                                                  *
 1.532E+04  1.92E+02                                                *
 1.406E+04  1.91E+02                                              *
 1.309E+04  1.90E+02                                            *
 9.995E-01  0.00E+00          *
1CONTIN VERSION 2DP (MAR 1984) ( CD-1  PACKAGE)  +++++++++++++++++++++++++++++++  CHOSEN SOLUTION
+++++++++++++++++++++++++++++++

        TEST DATA SET 1 - FOR CD PACKAGE

    ALPHA    ALPHA/S(1)    OBJ. FCTN.      VARIANCE     STD. DEV.   DEG FREEDOM    PROB1 TO REJECT    PROB2 TO REJECT
  1.16E-04     2.84E-03   3.21449E+02   7.66531E+01     1.379E+00       11.693           0.942              1.000

   ORDINATE    ERROR  ABSCISSA
  1.839E-01  2.5E-02  1.00E+00                                ..X..
 -2.127E-01  4.5E-02  2.00E+00          ....X....
  3.517E-01  2.8E-02  3.00E+00                                    ...X..
 -4.015E-01  2.8E-02  4.00E+00X...
  2.639E-01  3.0E-02  5.00E+00                                  ..X...
 -1.354E-01  3.7E-02  6.00E+00               ...X....
  7.405E-01  2.6E-02  7.00E+00                                                ..X
 -4.111E-01  3.3E-02  8.00E+00X..
 -1.148E-01  4.9E-02  9.00E+00               ....X....
  2.931E-02  5.1E-02  1.00E+01                     .....X....
 -4.113E-01  2.8E-02  1.10E+01X..
 -3.648E-01  2.7E-02  1.20E+01 ...X..
  6.917E-01  2.6E-02  1.30E+01                                            ..X...
  1.132E-01  3.9E-02  1.40E+01                             ...X...
  2.687E-01  4.5E-02  1.50E+01                                 ....X...
  4.083E-01  2.8E-02  1.60E+01                                     ...X..

                HELIX   BETA-SHEET    REMAINDER            SCALE FACTOR
 FRACTION            0.09        0.63        0.28                   1.000
 STANDARD ERROR      7.2E-03     2.2E-02     1.8E-02
```

OmpF WT pH=7

```
0PLOT OF DATA (O) AND FIT TO DATA (X).  ORDINATES LISTED ARE FIT VALUES.

   ORDINATE  ABSCISSA
 -5.923E+02  2.40E+02                    *
 -6.064E+02  2.39E+02                    OX
 -7.912E+02  2.38E+02                    *
 -1.093E+03  2.37E+02                    *
 -1.455E+03  2.36E+02                   XO
 -1.813E+03  2.35E+02                   XO
 -2.172E+03  2.34E+02                   XO
 -2.546E+03  2.33E+02                  *
 -2.839E+03  2.32E+02                  *
 -3.124E+03  2.31E+02                 *
 -3.493E+03  2.30E+02                 *
 -4.046E+03  2.29E+02                *
 -4.598E+03  2.28E+02                *
 -5.181E+03  2.27E+02               *
 -5.721E+03  2.26E+02              *
 -6.329E+03  2.25E+02             *
 -6.986E+03  2.24E+02            XO
 -7.676E+03  2.23E+02           XO
 -8.245E+03  2.22E+02          *
 -8.699E+03  2.21E+02         *
 -9.176E+03  2.20E+02         *
 -9.625E+03  2.19E+02 *
 -1.000E+04  2.18E+02*
 -1.017E+04  2.17E+02*
 -1.019E+04  2.16E+02*
 -1.005E+04  2.15E+02*
 -9.785E+03  2.14E+02 *
 -9.352E+03  2.13E+02  *
 -8.650E+03  2.12E+02    XO
```



1CONTIN VERSION 2DP (MAR 1984) (CD-1 PACKAGE) ++++++++++++++++++++++++++++++++ CHOSEN SOLUTION
++++++++++++++++++++++++++++++++

TEST DATA SET 1 - FOR CD PACKAGE

ALPHA	ALPHA/S(1)	OBJ. FCTN.	VARIANCE	STD. DEV.	DEG FREEDOM	PROB1 TO REJECT	PROB2 TO REJECT
4.47E-05	1.11E-03	9.58356E+01	5.14683E+01	1.150E+00	13.115	0.715	1.000

ORDINATE	ERROR	ABSCISSA
7.110E-01	7.8E-02	1.00E+00
-1.153E-01	1.0E-01	2.00E+00
6.941E-01	1.1E-01	3.00E+00
-2.895E-01	4.8E-02	4.00E+00
-2.270E-01	1.5E-01	5.00E+00
1.485E-01	6.3E-02	6.00E+00
1.055E+00	5.5E-02	7.00E+00
-1.032E+00	7.7E-02	8.00E+00
4.650E-02	9.9E-02	9.00E+00
-7.651E-01	9.9E-02	1.00E+01
-4.206E-01	4.6E-02	1.10E+01
-1.734E-01	5.7E-02	1.20E+01
5.545E-01	4.9E-02	1.30E+01
2.441E-01	6.5E-02	1.40E+01
2.769E-01	9.7E-02	1.50E+01
2.843E-01	1.1E-01	1.60E+01

	HELIX	BETA-SHEET	REMAINDER	SCALE FACTOR
FRACTION	0.12	0.88	0.00	0.992
STANDARD ERROR	9.5E-03	2.8E-02	3.8E-09	

REFERENCES

1. R. Darı´o Falcone, N. M. Correa, M. A. Biasutti and J. J. Silber, *Langmuir*, 2002, **18**, 2039-2047.
2. R. A. Boeckmann and A. Caflisch, *Biophysical J.*, 2005, **88**, 3191–3204.