Supplementary Information
for the article

Structure and Diffusive dynamics of Aspartate α-decarboxylase (ADC) liganded with D-Serine in aqueous solution

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1 Amber99SB-ILDN force-field parameters

All parameters given below are in the Gromacs format. We followed the Gromacs manual for adding the residues:

\url{http://www.gromacs.org/Documentation/How-tos/Adding_a_Residue_to_a_Force_Field}

1.1 Atom types, charges and bonded interactions

The lines below were added to aminoacids.rtp in the force field folder. Atom names in the pdb need to be adjusted accordingly before processing.

; New residue for standalone serine
[SSE]
[atoms]
N N3 -0.20654 1
H1 H 0.25303 2
H2 H 0.25303 3
H3 H 0.25303 4
CA CT 0.03897 5
HA HP 0.07843 6
CB CT 0.05876 7
HB1 H1 0.08320 8
HB2 H1 0.08320 9
OG OH -0.65637 10
HG HO 0.44926 11
C C 0.68350 12
OC1 O2 -0.68620 13
OC2 O2 -0.68620 14

[bonds]
N H1
N H2
N H3
N CA
CA HA
CA CB
CA C
CB HB1
CB HB2
CB OG
OG HG
C OC1
C OC2

[impropers]
CA OC1 C OC2

; New residue for pyruvoyl
[PYR]
[atoms]
1.1.1 Addition of hydrogens

This file is necessary, if hydrogens have to be added to the pdb structure. Please add the following lines to aminoacids.hdb.

```
SLE  6
  3  4  H  N  CA  CB
  1  5  HA  CA  N  CB  C
  2  6  HB  CB  CA  CG
  1  5  HG  CG  CB  CD1  CD2
  3  4  HD1  CD1  CG  CB
  3  4  HD2  CD2  CG  CB
PYR  1
  3  4  H3  C3  C2  O2
```

1.1.2 Translation of atom names in pdb to force-field names

Please add this to the file aminoacids.arn.

```
SSE   0   0C2
SSE   OXT  0C1
```
Figure S2: Histograms for the average $C_\alpha$-$C_\alpha$ distances for the individual subunits between HIS-21 – GLY-24 (top) and between TYR-22 – GLY-24 (bottom) for apo-ADC (blue) and D-Serine liganded ADC (orange).

Figure S3: RMSD of the MD simulation allowing for an estimation of the duration of the equilibration.
Figure S4: DSSP (hydrogen bond estimation algorithm) calculation for the trajectories of apo ADC and the ADC D-Serine complex at 310 K. The chains are separated by dashed red lines.

Figure S5: Distance between HIS-21 and GLY-24: the loop opens up as a result of ligand binding which involves a change in distance between Cα atoms of the terminal residues HIS-21 and GLY-24 from $\approx 7.5$ to $10.5$ Å (displacement by $\approx 3$ Å).
3 Additional fit parameters for neutron time-of-flight spectra (IN5)

The figure S6 reports the energy resolution function of IN5 for this experiment, and the figures S7, S8, S9 report the additional fit parameters according to equation 2 of the main article. The apparent elastic contribution $I_\delta$ displayed in figure S8 is reasonably consistent with a thermal Debye-Waller (or Lamb-Mössbauer) factor of proteins that are immobile on the observation time scale of the IN5 experiment given by its energy resolution.

Figure S6: Energy resolution of the time-of-flight spectrometer IN5 for the employed cylindrical sample geometry with 22 mm cylinder diameter, for $q = 0.6 \, \text{Å}^{-1}$, measured using Vanadium foil as the sample (symbols). The solid lines represent Gaussian functions and their sum fitted to the measured data. The sum of Gaussians is used to analytically describe the resolution function.

In the legends, ADC-lig. denotes ADC liganded, i.e. complexed, with D-Serine.

Figure S7: Summary of the fit parameters for the intensities of the Lorentzians $\mathcal{L}(\gamma)$ (top) and $\mathcal{L}(\Gamma)$ (bottom) associated with the proteins in the samples, for the samples as assigned in the legends. The error bars account for the $1 \cdot \sigma$ confidence bounds of the fits.
Figure S8: Fit results for the elastic contribution $I_\delta$ in equation 2 of the main article (bottom), for the samples as assigned in the legends.

Figure S9: Width $\Gamma_{IN5}$ of the Lorentzian accounting for fast internal diffusive motions observed on IN5 (equation 2 of the main part) for D-Serine liganded ADC at different temperatures (symbols) and fits using the jump diffusion model (equation 5 of the main part), the fit parameters being reported in the legend.
4 Dynamic light scattering

Using DLS, a time autocorrelation function was measured over the angular range 30-150 ° (figure S10). The correlation function for a monodisperse sample is given as

\[ g^1(q,t) = a \exp(-2\Gamma t). \]  

(1)

In case of more than one populations of clusters with different diffusion coefficients, the following equation is used as the fit function:

\[ g^1(q,t) = a_1 \exp(-2\Gamma_1 t) + a_2 \exp(-2\Gamma_2 t) + a_3 \exp(-2\Gamma_3 t) + ..., \]  

(2)

where \( \Gamma \) is the decay rate and \( t \) the time. The first order autocorrelation function was treated as a monoexponential decay (equation 1) in order to extract the decay rate.

\( \Gamma \) can then be plotted versus \( q^2 \) which follows Fickian diffusion,

\[ 2\Gamma = D_t q^2, \]  

(3)

where \( D_t \) is the translational diffusion coefficient and \( q \) is the momentum transfer defined as

\[ q = \frac{4\pi n_0}{\lambda} \sin \left( \frac{\theta}{2} \right). \]  

(4)

where \( \theta \) is the scattering angle, \( n_0 \) the refractive index of the sample, and \( \lambda \) the wavelength of the incident beam. Due to the linear relation of \( \Gamma \) and \( q^2 \) (equation 3) a linear fit of the \( q \)-dependence gives the long-time translational diffusion coefficient \( D_t \) (figure S11).

Figure S10: Top: DLS data recorded at \( \Theta = 120^\circ \) and single-exponential fit (equation [1]) of the autocorrelation function \( g^{-1} \) for D-Serine liganded ADC at \( c_p = 15 \text{ mg/mL} \) and \( T = 298 \text{ K} \) versus correlation time \( t \). Bottom: DLS data recorded at 70, 80, 100, 120, 140° for the same sample. Symbols represent the data whereas the solid line represents the fit.

Figure S11: Linear fit of the decay rate obtained from the DLS data for D-Serine liganded ADC (squares) and apo-ADC (circles) at 15 mg/mL protein concentration.
5 Radial hydrogen density distribution calculated from the pdb files

Figure S12: Radial hydrogen density distribution calculated from the respective pdb files for ADC tetramers without ligand, ADC dimers liganded with D-Serine, and ADC tetramers liganded with D-Serine, respectively, as assigned in the legend.

6 Apparent hydrodynamic radius and cluster size assuming compact spherical clusters

Figure S13: Apparent hydrodynamic radius $R_{h,app}$ calculated using equation 9 from the main part of the manuscript. The radius calculated from the QENS results (cf. assignment in the legend) only represents an apparent radius – which largely underestimates the actual radius –, because the rotational and translational contributions to the diffusion coefficient have not been separated (cf. figure S14). The hydrodynamic radius calculated from the HYDROPRO (denoted ‘HYD’ in the legend) and DLS results is exact, because these methods directly provide the translational diffusion coefficient. The different solvent viscosities in the presence and absence of excess D-Serine have been taken into account (cf. table S1 in this Supporting Information). Since the apparent radius underestimates the actual radius, a crowding-induced enhanced cluster formation can be deduced when comparing to the dilute limit (HYDROPRO and DLS).
Figure S14: The considerations on the cluster hydrodynamic radius (figure S13) can be carried even further: Assuming compact spherical clusters and the radial hydrogen density distribution $\rho_H(r) = 4r_0\pi r^2 \Theta(R_{h,\text{cluster}} - r)$ with the normalization factor $r_0$ and Heaviside step function $\Theta$ (which reasonably approximates a distribution as in figure S12), the theoretical translational $D_t$ and apparent $D = D(D_t, D_r)$ diffusion coefficients can be calculated as explained in the main part of the manuscript. To this effect, the cluster hydrodynamic radius $R_{h,\text{cluster}}$ has been assumed to follow the simple volume scaling $R_{h,\text{cluster}} = (n \cdot R_h^3)^{1/3}$, where $n$ is the number of tetramers in the cluster and $R_h$ the tetramer hydrodynamic radius from HYDROPRO. The above figure is identical to figure 8 in the main part of the article, but contains additional dash-dotted and dotted lines representing the cluster $D$ and $D_t$, respectively, assuming $n = 10$ tetramers for the apo form of ADC and $n = 58$ tetramers for ADC liganded with D-Serine. With these crude assumptions, the experimental values for $D$ measured with QENS can be described. The corresponding cluster radii in these assumptions amount to $R_{h,\text{cluster}} = 63.8\,\text{Å}$ for the apo form and $127.0\,\text{Å}$ for the liganded form.
7 Tabled HYDROPRO input and output parameters and experimental diffusion coefficients

Table S1: HYDROPRO input and output parameters for apo-ADC (pdb 1AW8) and D-Serine liganded ADC (pdb 7A8Y from this work) structures, respectively, for the temperatures $T = 280$, 295 and 310 K. The hydrodynamic radius $R_h$ was not directly taken from the HYDROPRO output, but calculated from the HYDROPRO output for the translational diffusion coefficient $D_t$ via the Stokes-Einstein relation (equation (9) of the main text) for $\phi = 0$.

| PDB file (tetramer) | ADC-APO | ADC-APO | ADC-APO | ADC-LIG | ADC-LIG | ADC-LIG |
|---------------------|---------|---------|---------|---------|---------|---------|
|                     | 1AW8    | 1AW8    | 1AW8    | 7A8Y    | 7A8Y    | 7A8Y    |
| Radius of elements in primary model (Å) | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.9 |
| Temperature (K)     | 280     | 295     | 310     | 280     | 295     | 310     |
| Solvent viscosity (pure D2O) (poise) | 0.01830 | 0.01175 | 0.00830 | 0.01976 | 0.01270 | 0.00896 |
| Molecular weight (Da)   | 50391   | 50391   | 50391   | 50811   | 50811   | 50811   |
| Solute partial specific volume (cm$^3$/g) | 0.702 | 0.702 | 0.702 | 0.702 | 0.702 | 0.702 |
| Solution density (g/cm$^3$) | 1.110 | 1.110 | 1.110 | 1.110 | 1.110 | 1.110 |
| Translational diffusion coefficient $D_t(\phi=0)$ (cm$^2$/s) | 3.784E-07 | 6.209E-07 | 9.236E-07 | 3.163E-07 | 5.188E-07 | 7.721E-07 |
| Radius of gyration (cm) | 2.257E-07 | 2.257E-07 | 2.257E-07 | 2.446E-07 | 2.446E-07 | 2.446E-07 |
| Hydrodynamic radius $R_h$ (cm) | 2.962E-07 | 2.962E-07 | 2.962E-07 | 3.281E-07 | 3.279E-07 | 3.282E-07 |
| Volume (cm$^3$) | 8.235E-20 | 8.235E-20 | 8.235E-20 | 9.476E-20 | 9.476E-20 | 9.476E-20 |
| Rotational diffusion coefficient ($D_r(\phi=0)$) (s$^{-1}$) | 3.174E+06 | 5.208E+06 | 7.747E+06 | 2.124E+06 | 3.484E+06 | 5.185E+06 |

Table S2: Diffusion coefficients obtained from QENS, DLS and HYDROPRO at $T = 295$ K, and hydrodynamic radius calculated from the DLS results using the Stokes-Einstein relation. The errors denote 67% confidence bounds on the fits and do not account for systematic errors arising from the choice of the model. Note that the hydrodynamic radii $R_h$ have been calculated from $D_t$ accounting for the different viscosities in the presence and absence of excess D-Serine in the solvent (cf. table 1 above).

|        | QENS $D(\phi=0.09)$ | DLS $D_t(\phi \approx 0)$ | HYDROPRO $D_t(\phi=0)$ | DLS $R_h$ |
|--------|---------------------|---------------------------|-------------------------|-----------|
| ADC-APO | (2.82±0.03) Å$^2$/ns | (4.30±0.03) Å$^2$/ns | 6.21 Å$^2$/ns | (4.28±0.03) nm |
| ADC-LIG | (1.39±0.02) Å$^2$/ns | (4.05±0.02) Å$^2$/ns | 5.19 Å$^2$/ns | (4.20±0.02) nm |

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