Modulation of Acetylcholinesterase Activity Using Molecularly Imprinted Polymer Nanoparticles

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**Supplementary Data Tables**

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**Table S2.** Affinity coefficient (KD) of the synthesized MIP nanoparticles upon interaction with EeAChE enzyme measured using SPR.

**Table S3.** Values obtained from Michaelis-Menten plot for FRF-MIP.



Fig. S1. Solid phase synthesis of MIP nanoparticles.





**Fig. S2.** Size distribution by intensity (top) and correlograms (bottom) for YWA-MIPs synthesized in this work (for the size and distribution values, see Table S1).





**Fig. S3.** Size distribution by intensity (top) and correlograms (bottom) for FRF-MIPs synthesized in this work (for the size and distribution values, see Table S1).



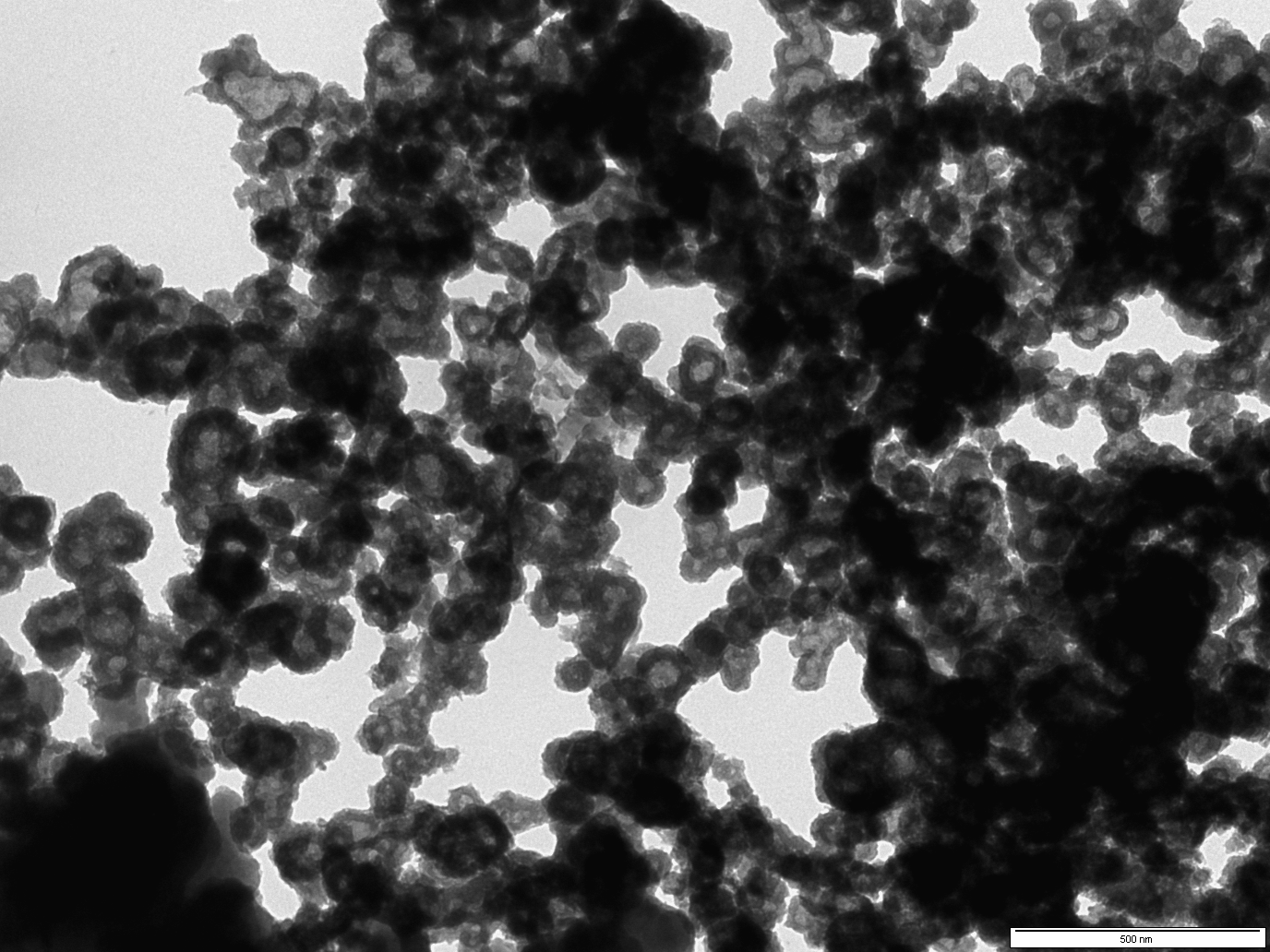


**Fig. S4.** Size distribution by intensity (top) and correlograms (bottom) for LAL-MIPs synthesized in this work (for the size and distribution values, see Table S1).





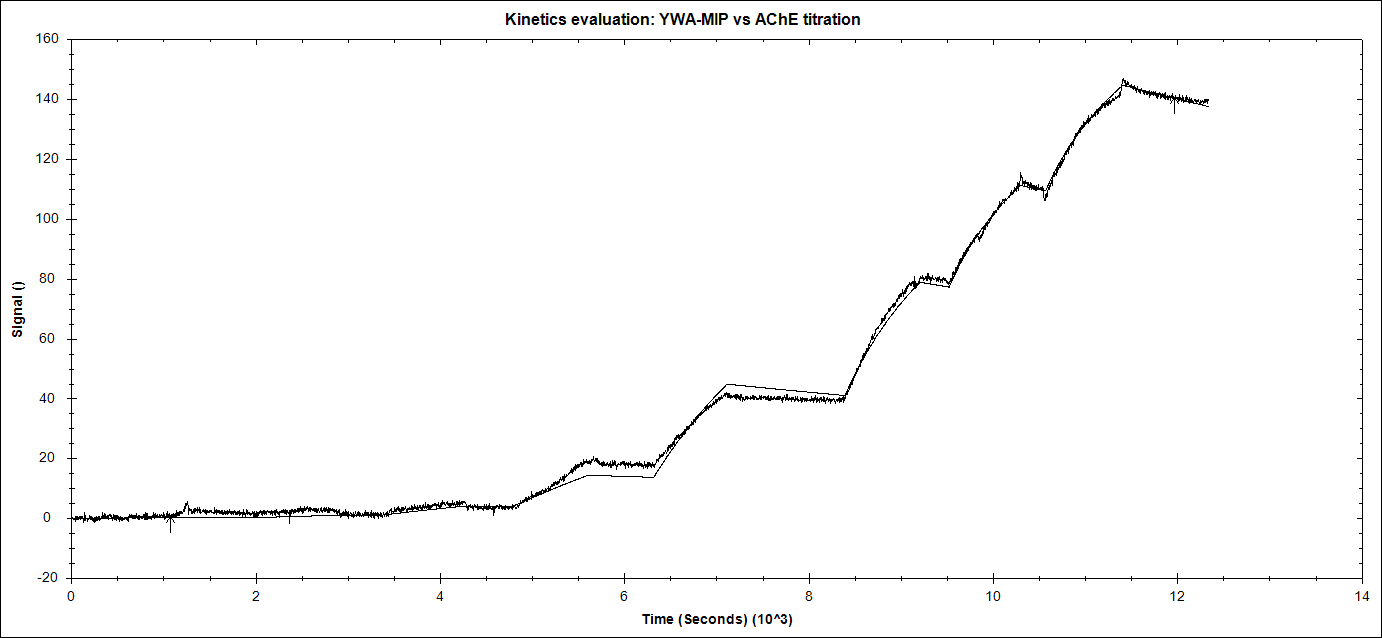
**Fig. S5.** Size distribution by intensity (top) and correlograms (bottom) for FGE-MIPs synthesized in this work (for the size and distribution values, see Table S1).



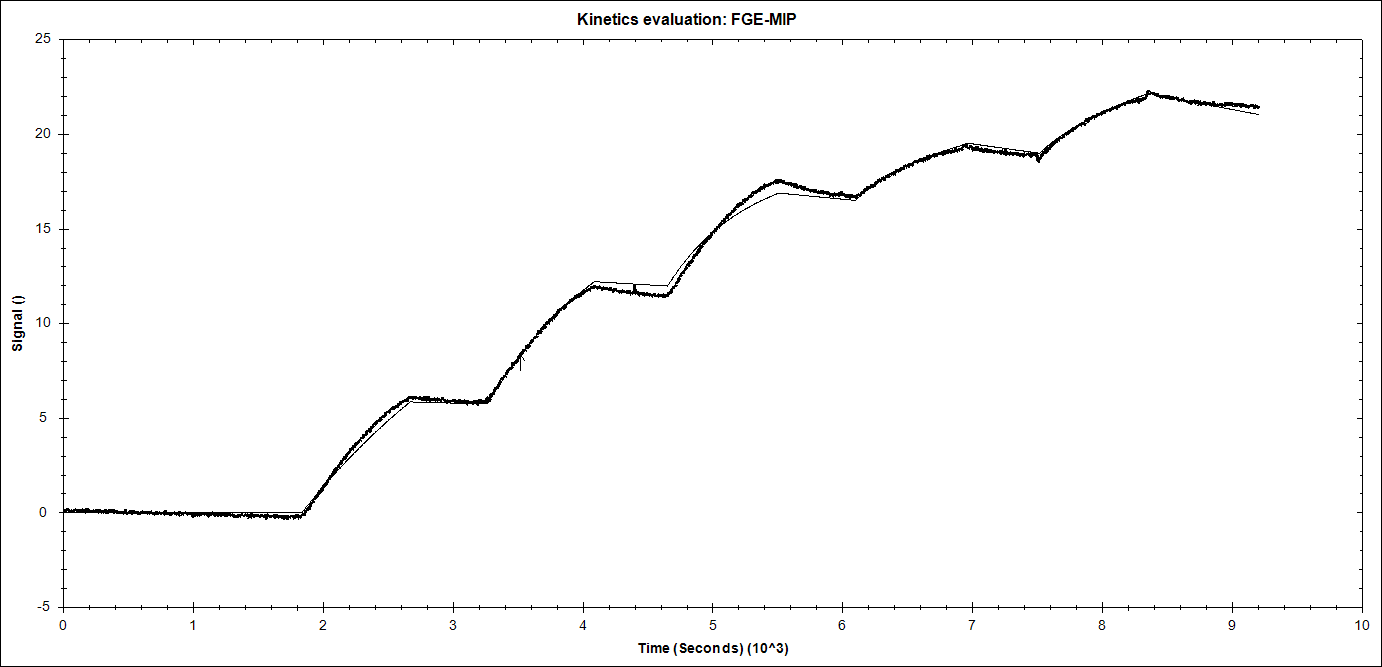
**Fig. S6.** Typical TEM image of the YWANFAR-specific MIP NPs specific for AChE.

**Table S1**: Size values and distribution for the nanoMIPs measured using DLS.

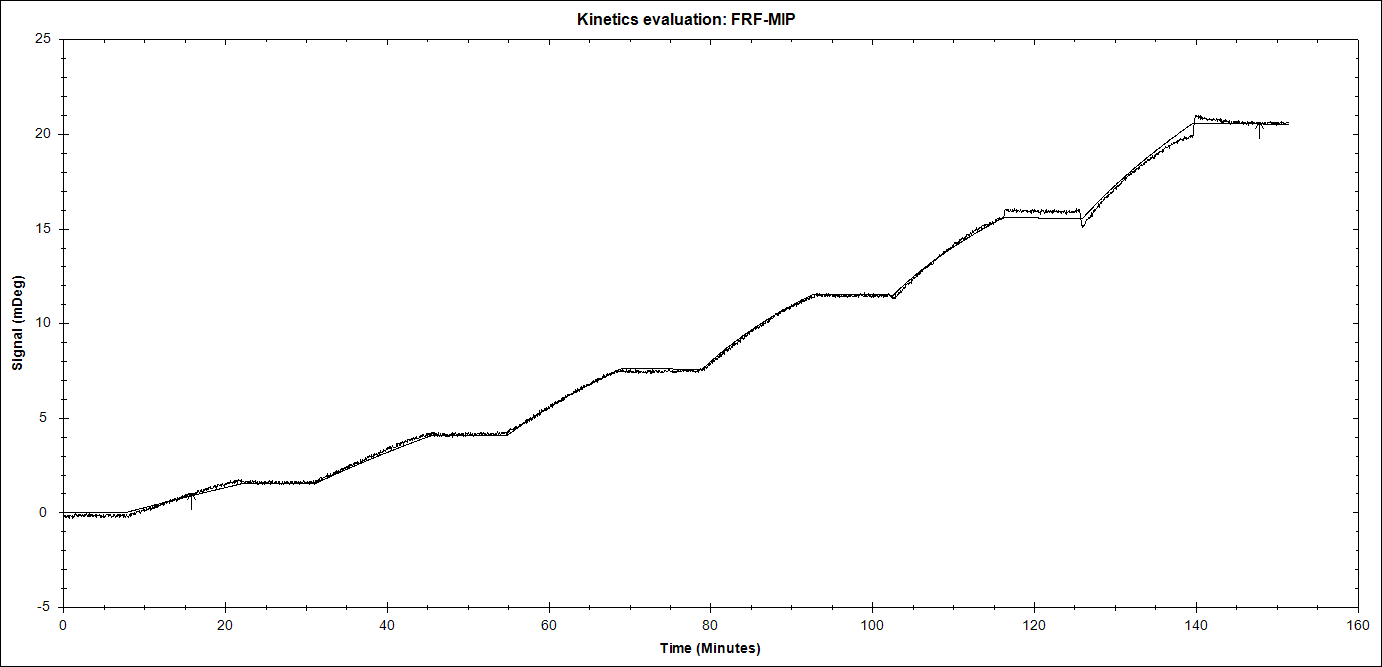
|  |  |  |  |
| --- | --- | --- | --- |
| **MIP** | **Size by intensity (d. nm)** | **Pdi** | **Number mean (d. nm)** |
| LAL | 408.4 ± 20.52 | 0.439 ± 0.056 | 393.6 ± 18.87 |
| YWA | 189.5 ± 10.52 | 0.304 ± 0.032 | 44.81 ± 2.86 |
| FRF | 484.3 ± 1.818 | 0.102 ± 0.047 | 431.1 ± 7.04 |
| FGE | 163.5 ± 9.381 | 0.140 ± 0.016 | 101.5 ± 6.76 |



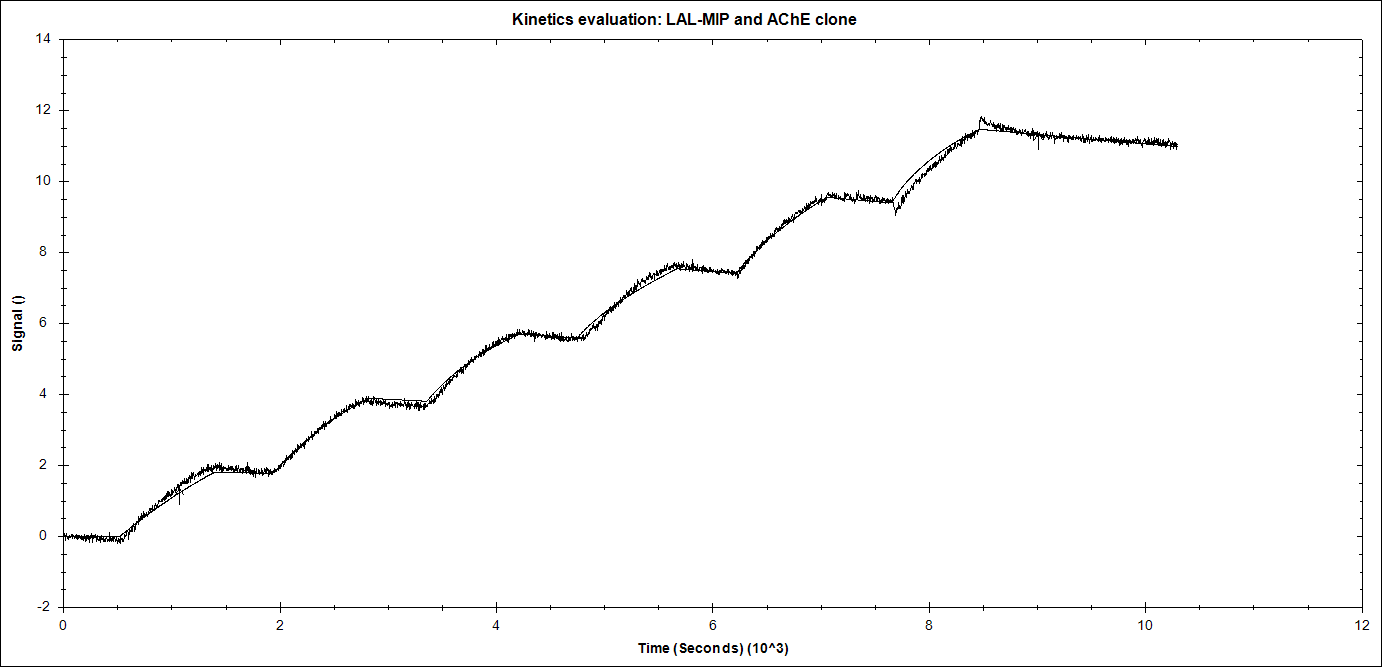
**Fig. S7**. SPR sensorgrams showing response of YWA-MIPs to injections of different concentrations of AChE. A kinetic titration injection strategy was employed for these experiments due to the difficulty of surface regeneration. AChE was injected at 5 different concentrations from 0.1 nM to 1 µM and allowed to associate and dissociate for 14 min and 5 min respectively, before a final dissociation of 120 min (not shown). All data were reference subtracted against a control polymer of the same monomer composition, and fit to a 1:2 interaction model using Tracedrawer 1.8 software.



**Fig. S8.** SPR sensorgrams showing response of FGE-MIPs to injections of different concentrations of AChE. A kinetic titration injection strategy was employed for these experiments due to the difficulty of surface regeneration. AChE was injected at 5 different concentrations from 0.1 nM to 1 µM and allowed to associate and dissociate for 14 min and 5 min respectively, before a final dissociation of 120 min (not shown). All data were reference subtracted against a control polymer of the same monomer composition, and fit to a 1:2 interaction model using Tracedrawer 1.8 software.



**Fig. S9.** SPR sensorgrams showing response of FRF-MIPs to injections of different concentrations of AChE. A kinetic titration injection strategy was employed for these experiments due to the difficulty of surface regeneration. AChE was injected at 5 different concentrations from 0.1 nM to 1 µM and allowed to associate and dissociate for 14 min and 5 min respectively, before a final dissociation of 120 min (not shown). All data were reference subtracted against a control polymer of the same monomer composition, and fit to a 1:2 interaction model using Tracedrawer 1.8 software.



**Fig. S10.** SPR sensorgrams showing response of LAL-MIPs to injections of different concentrations of AChE. A kinetic titration injection strategy was employed for these experiments due to the difficulty of surface regeneration. AChE was injected at 5 different concentrations from 0.1 nM to 1 µM and allowed to associate and dissociate for 14 min and 5 min respectively, before a final dissociation of 120 min (not shown). All data were reference subtracted against a control polymer of the same monomer composition, and fit to a 1:2 interaction model using Tracedrawer 1.8 software.

**Table S2.** Affinity coefficient (KD) of the synthesized MIP nanoparticles upon interaction with EeAChE enzyme measured using SPR.

|  |  |  |
| --- | --- | --- |
| Epitope used as template | KD, nM | Chi2 |
| YWANFAR (YWA-MIP) | 12.0 | 4.03 |
| QVTIFGESAGAASVGM-  HLLSPDSRPK (FGE-MIP) | 78.6 | 0.06 |
| FRFSFVPV (FRF-MIP) | 0.40 | 0.03 |
| LALQWVQDNIHFFGGNPK (LAL-MIP) | 2.20 | 0.02 |

**Fig. S11.** Direct measure of substrate conversion by enzyme alone and in the presence of FRF-MIP.

**Fig. S12.** Circular dichroism spectra of AChE with increasing concentration of YWA-MIP. Dashed lines correspond to control measurements of MIP without protein. Note that the nanoMIP materials appear to be inert spectroscopically.



**Fig. S13.** Michaelis-Menten plot obtained for FRF-MIP. Error bars indicate SD. N=3

**Table S3.** Values obtained from Michaelis-Menten plot for FRF-MIP.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **FRF-MIP- AChE** | **Malathion-AChE** | **Prevention** | **Regeneration** | **AChE** |
| Michaelis-Menten |  |  |  |  |  |
| Best-fit values |  |  |  |  |  |
| Vmax | 143.9 | 25.01 | 45.12 | 34.92 | 119.7 |
| Km | 0.5378 | 0.674 | 0.5301 | 1.147 | 0.3928 |
| Std. Error |  |  |  |  |  |
| Vmax | 6.829 | 1.315 | 4.506 | 4.047 | 3.342 |
| Km | 0.06444 | 0.08344 | 0.1343 | 0.2637 | 0.03039 |
| 95% CI (profile likelihood) |  |  |  |  |  |
| Vmax | 130.8 to 159.4 | 22.45 to 28.15 | 36.93 to 57.09 | 28.23 to 45.9 | 112.7 to 127.5 |
| Km | 0.4222 to 0.6895 | 0.5203 to 0.8828 | 0.3127 to 0.9282 | 0.7399 to 1.902 | 0.3313 to 0.4664 |
| Goodness of Fit |  |  |  |  |  |
| Degrees of Freedom | 12 | 12 | 12 | 12 | 12 |
| R square | 0.9863 | 0.9867 | 0.9372 | 0.9714 | 0.9924 |
| Absolute Sum of Squares | 295.1 | 7.575 | 131.4 | 25.55 | 110.8 |
| Sy. x | 4.959 | 0.7945 | 3.309 | 1.459 | 3.038 |
| Constraints |  |  |  |  |  |
| Km | Km > 0 | Km > 0 | Km > 0 | Km > 0 | Km > 0 |
|  |  |  |  |  |  |
| Number of points |  |  |  |  |  |
| # of X values | 14 | 14 | 14 | 14 | 14 |
| # Y values analyzed | 14 | 14 | 14 | 14 | 14 |